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ERRATA

- Page 38, article by Tapke, last line, instead of "controls loose smut of wheat reducing yields" read "controls loose smut of wheat without reducing yields."
- Page 55, second abstract title change "tubes" to "tubers."
- Page 56, first abstract title "J. I. Jauritzen" should read J. I. Lauritzen.
- Page 90, line 5, instead of "one plant" read "all the descendants of one plant."
- February number, table of contents on cover, change pagination as follows: 74 to 75, 85 to 87, 97 to 100, and 100 to 101.
- February number, omit "Phytopathological notes 103" on cover.
- Page 231, line 10 from bottom, change Actinomyceetes to Actinomyces.
- July number, table of contents on cover, change authors of sixth article to Charles A. Kofoid, Henry H. P. Severin, and Olive Swezy.
- Page 302, name of author, change "B" to "C."
- Page 386, line 12, foot note reference number 1 refers to footnote at end of article on page 391.
- Page 389, first column of table, first line change "Phythium" to Pythium.
- Page 391, (1) Buttner, etc. change "otwas her" to "otwas liber." Footnote should go at foot of page 386.
- December number cover. Next to last title in contents, insert "selected" after "isolating."
- Page 520, line 10, change "more variable conidia" to "less variable conidia".

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PHYTOPATHOLOGY

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NUMBER 1

III. SEPTORIA DISEASE OF RYE, BARLEY AND CERTAIN GRASSES.

GEORGE F. WEBER

WITH NINE FIGURES IN THE TEXT

SEPTORIA LEAF BLOTCH OF RYE¹

INTRODUCTION

A disease was found on the leaves of rye in the experimental plot at the Hill Farm of the University of Wisconsin. On examination it was found to be caused by *Septoria secalis* Prill. and Del. The leaves were the only part of the rye plant attacked. The lesions were rather irregular in outline ranging from small, almost circular spots to larger areas often involving the whole leaf blade. The center of the spots was yellowish to grayish-white as described by Kirchner (6) in Germany. In many instances the yellow was entirely lacking and large portions of the leaf were grayish-white and thickly speckled with pycnidia. Leaves were found infected from the time of the appearance of growth in the spring until harvest. After blossoming time the infected leaves on the culm showed more bleaching than did the infected leaves earlier in the season. It was reported from different parts of France by Prillieux and Delacroix (9) in 1889, who described it on the leaves of *Secale cereale*. In the United States it has been reported from Virginia, West Virginia, Illinois, and at this time from Wisconsin. It has not been found to be of economic importance in this vicinity even though 100 per cent of plants were infected. It has been reported only on rye.

¹ This is the third and last of three articles by the author on Septoria diseases of cereals and certain grasses. The first appeared in *Phytopathology*, Number 10, Volume XII, the second in Number 12, Volume XII.

CAUSAL ORGANISM

The causal organism was found, described, and named *Septoria secalis* by Prillieux and Delacroix (9) in 1889. The fungus grows readily in culture. The mycelium is hyaline, septate, 1 to 2 μ in diameter, branched, and whitish in colonies. The pycnidia are subepidermal, black, globose to subglobose, smooth, 85 to 130 μ in diameter. The pycnosporos (Fig. 7, E) are hyaline, 3-septate, usually curved, occasionally straight, and rounded at the ends. The content of the pycnosporos is homogeneous or slightly guttulate. The pycnosporos commonly measure 2.7 by 35 μ , extremes 2 to 3.5 by 25 to 49 μ . The pycnosporos were exuded by soaking the leaf tissue containing pycnidia in water for from ten to fifteen minutes. They were then suspended in water and placed on a potato-dextrose agar culture for germination. Germination tubes were found after from 6 to 8 hours. Transfers were then made to culture tubes. In these tubes abundant mycelium was found but no conidia or perithecia were formed. Pycnosporos suspensions obtained in the manner described were used as inoculum and sprayed on a number of rye plants with an atomizer. The plants were immediately placed in a moist chamber for 48 hours and then placed on a greenhouse bench. After two weeks a large number of the leaves showed the disease with pycnidia present. A microscopical examination of the pycnosporos showed that they were identical with those collected on rye plants in the field. A large number of cross inoculations were made on other cereals and certain grasses. The summary of these results is shown in table 1 and represented diagrammatically in figure 1.

As seen from table 1, and figure 1, *Septoria secalis* was restricted to rye. It would not infect any of the forty species and varieties of cereals and grasses tested, as shown by table 1.

SUMMARY

1. The disease of rye caused by *Septoria secalis* Prill. and Del. has been previously reported only from France. It has been found to occur more or less sparingly in Virginia, West Virginia, Illinois, and Wisconsin.

2. The disease has been found to be of no economic importance.

3. The causal fungus grows readily in artificial culture on potato-dextrose agar.

4. The pycnosporos are hyaline, septate, and most commonly measure 2.7 by 35 μ .

5. The fungus is pathogenic on the leaves of rye and apparently limited to that host.

SEPTORIA LEAF BLOTCH OF BARLEY

A disease of barley caused by *Septoria passerinii* Sacc. was found in the vicinity of Madison, Wisconsin, in 1920. Leaves of *Hordeum vulgare pallidum* on the Hill Farms of the University of Wisconsin were found heavily attacked. The lesions were very indefinite in outline, there

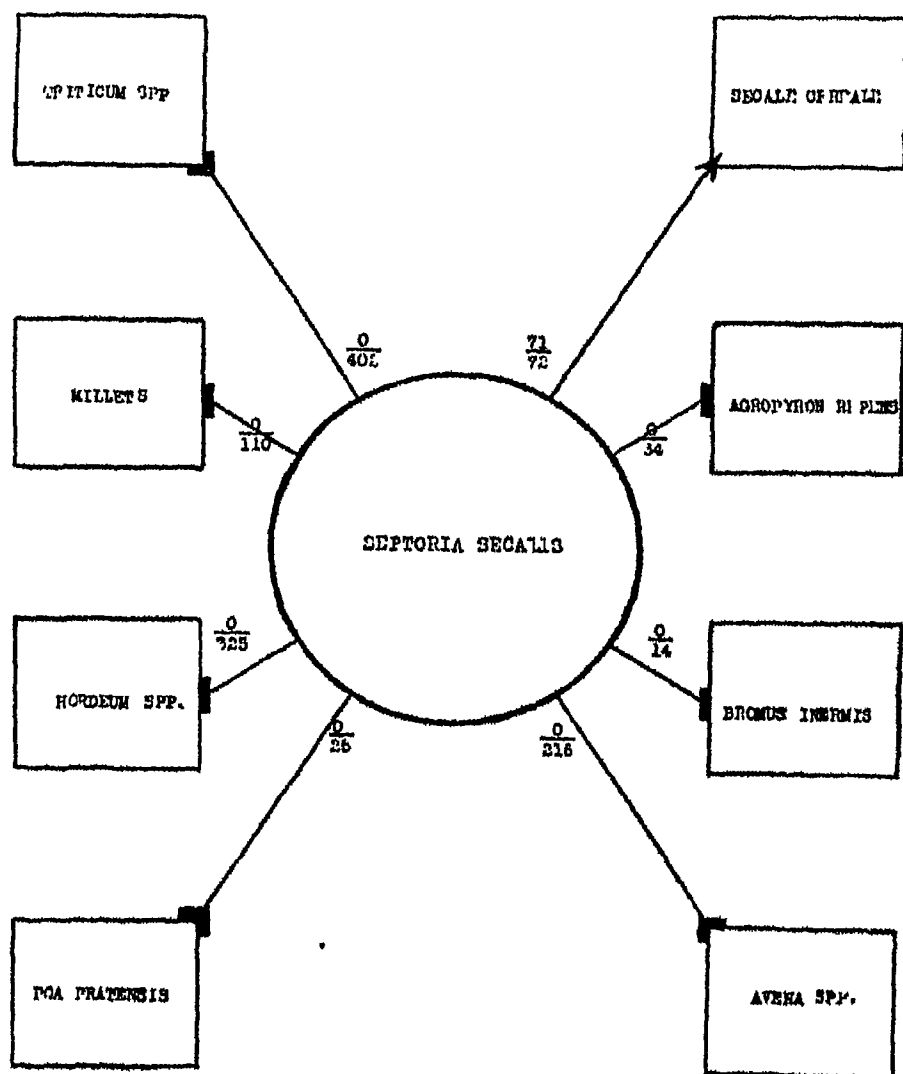


FIG. 1.—Diagram summarizing the results of inoculation experiments with *S. secalis*. Plants named in rectangles were inoculated with water suspensions of pycnosporos from rye. The denominator and numerator of the fractions designate the number of leaves inoculated and leaves infected respectively. Arrow indicates infection.

being no definite line of demarkation separating the diseased and healthy tissue but rather a gradual blending from the yellowish diseased area to the normal green of the healthy parts of the leaves. Pycnidia were fairly prominent as miniature black specks on the yellow area. They were found also though not always in a mature state in the green tissue

surrounding the yellowish parts. Inoculation experiments were conducted in field plots at the University of Wisconsin and the following symptoms of the disease were produced. The plants appeared normal until about the ninth or tenth day after inoculation at which time rather large, more or less irregular leaf areas began to fade and become yellowish. The color changed rapidly and on approximately the third day after the green color began to fade, small black, speck-like pycnidia were noticeable. After twelve or thirteen days the pycnidia were fairly prominent and in cases where the whole leaf had been inoculated it turned yellow rapidly, drooped and began to die and turn brown. Inoculation experiments out-of-doors especially in the fall, and those in the greenhouse did not produce quite the same symptoms of the disease. In the plots the green color in the lesion appeared to be retained somewhat longer after inoculation than was the case in the greenhouse. The periods of incubation remained the same, however, as the disease was reproduced under both conditions in approximately the same time. In the leaves on the plants out-of-doors the pycnidia were sometimes found in unfaded green areas of the leaf. This disease has been reported from Italy by Passerini (7) in 1879, and from the United States by Johnson (5) in 1916. In 1920 Passerini found the disease on *Hordeum murinum* in the vicinity of Parma. Johnson (5) found it in 1914, 1915, 1917, and 1918 on a large number of species and varieties of barley in North Dakota, South Dakota, and Minnesota. He did not make inoculation experiments and since the pycnosporos were very similar to those of *Septoria graminum* Desm. he suggested that they might be the same. In the vicinity of Madison, Wisconsin, the kernels were not affected even though all or nearly all of the leaves were heavily infected.

Passerini (7) named the causal organism *Septoria murina*. Saccardo and Trotter (11) observed the disease on leaves of *Hordeum vulgare* and found that the causal organism was very much like *Septoria murina* Pass. Saccardo (10) in compiling his *Sylloge Fungorum* found that von Thümen had already described a disease on Iris as *S. murina* and consequently he changed *S. murina* Pass. to *S. passerinii*, by which name the fungus has since been known. This disease was reported in the Plant Disease Survey (8) to occur in the United States, but to be of no economic importance.

Morphology of the Fungus

Mycelium.—In culture, very scant, hyaline, branched, septate, 1-2 μ in diameter.

Conidia.—In artificial culture the conidia are cylindrical, straight or slightly curved, rounded at the ends, hyaline, thin walled, 3-septate.

The contents are homogeneous or slightly guttulate. The conidia are indistinguishable from the pycnospores, 1.7 to 3 by 2.3 to 4.6 μ , averaging 2.3 by 3.4 μ .

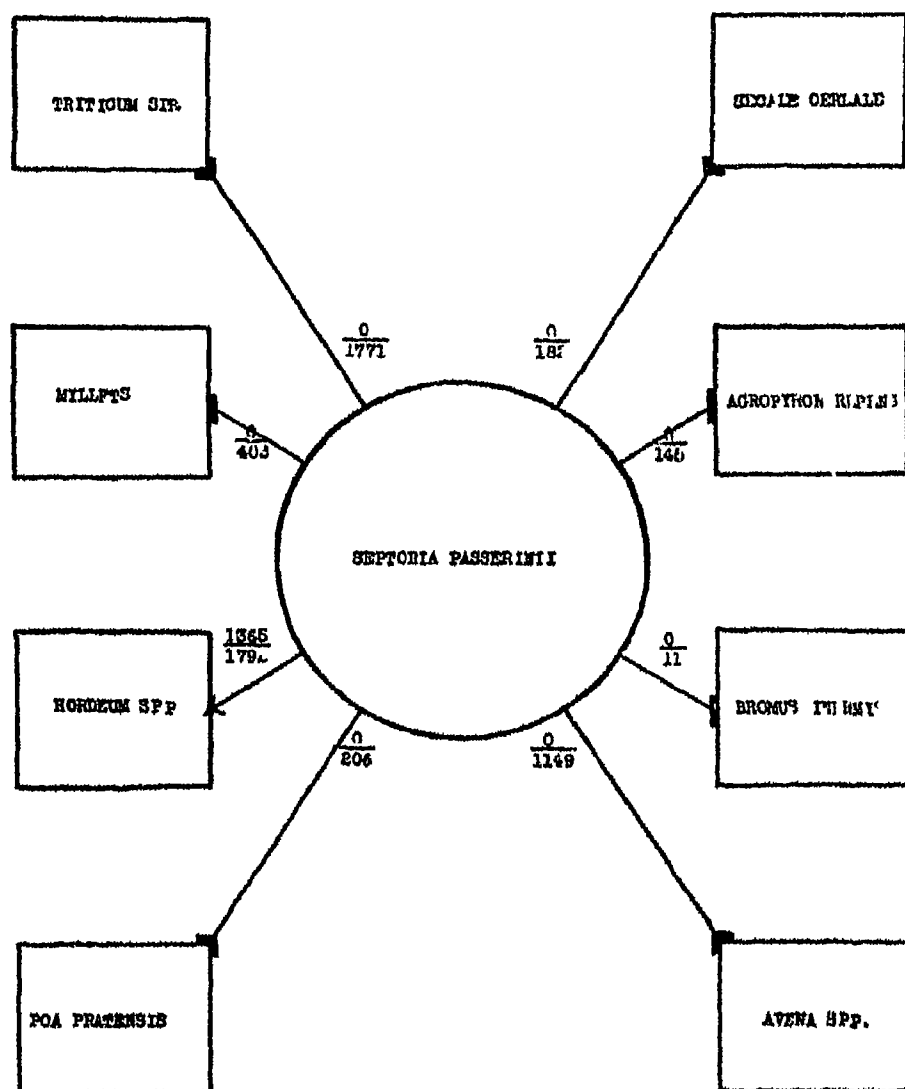


FIG. 2.—Diagram showing the fungus used as inoculum (in the circle), and the groups of host plants (in the rectangles). Denominators show the number of leaves inoculated, the numerators the number of leaves infected.

Pycnidia.—The pycnidia are subepidermal, black, smooth, globose to subglobose, 80 to 150 μ in diameter, wall pseudoparenchymatous, 1 to 3 cells thick. The ostiole is oval to elliptical, smooth, under stomata.

Pycnospores.—(Fig. 7, II). Same as conidia. Pycnospores exuded from pycnidium in long, fine, serpentine threads. Very slightly pinkish in mass.

Cultural studies and spore germination.—The pycnospores were secured from the leaves of infected barley plants by soaking pieces of these leaves bearing pycnidia in water for five or ten minutes. The exuded pycnospores suspended in water were used to inoculate solidified potato-dextrose agar poured plates. The medium when solidified in petri dishes was almost transparent. Previous to germination the pycnospores first enlarged, the septations became more prominent and numerous conidia were developed at the septa. These conidia increased in size until they were very similar to the original pycnospore. They in turn germinated by developing other conidia and after 72 hours a small colony was formed, made up almost entirely of conidia. There was a small amount of mycelium within the colony but none on the surface. The colony was raised, smooth, pink and circular. Transfers were made to potato-agar slants, and pinkish, raised, wrinkled growths were subsequently produced. These colonies did not remain pinkish but instead began to darken around the edge of the growth. After three or four weeks the whole surface of the colony became black, shiny, wrinkled and more or less leathery. The pycnospores and conidia germinated well in water, germinating most commonly from the end cells but occasionally from either of the others.

Pathogenicity

Pycnospore isolations were made from diseased leaves. Inoculation experiments were conducted which resulted in the reproduction of the disease after about two weeks showing that the fungus was pathogenic on the leaves of barley. It did not attack the hulls of the kernels, however. Both leaf sheaths and leaf blades were very susceptible. The pycnidia were more prominent on the sheaths and they were always distinctly arranged in rows parallel to the vascular strands. At Madison, Wisconsin, the fungus lived over winter in a manner similar to *S. tritici* on wheat. More than 90 per cent of the pycnospores collected in the field in April, 1922, from barley plants which had been heavily infected in September, 1921, germinated.

A series of cross inoculations were made in order to determine whether or not this fungus was pathogenic on wheat and other cereals. The inoculations were made on a plot containing about forty species and varieties of cereals and grasses. The inoculum consisted of a water suspension of pycnospores from infected barley leaves. The plants were sprayed with the inoculum by an atomizer and immediately covered with paper bags for two or three days. The results of these experiments are given in table 6 and graphically shown in figure 2.

The inoculations gave negative results on all host plants tried except species and varieties of *Hordeum*. On the members of this genus infection was rather uniform. The disease was produced in 1365 of the 1792 leaves which were inoculated.

SUMMARY

1. The Septoria leaf-blotch of barley previously reported from Italy, and from North Dakota, South Dakota, and Minnesota in the United States has been found in Wisconsin.

2. The causal fungus has been found pathogenic only on species and varieties of *Hordeum*. It is of no economic importance at the present time.

3. The causal organism has been found to be *Septoria passerinii* Sacc.

4. Pycnospores measure 1.7-3 by 23-46 μ .

5. The organism grows readily on potato-dextrose agar, forming flesh colored colonies of conidia, becoming black with age.

SEPTORIA LEAF BLOTCH OF QUACK GRASS (AGROPYRON REPENS)

In connection with the investigations of the Septoria diseases of cereals conducted by the writer, *Septoria agropyri* Ell. and Ev. on *Agropyron repens*, *S. bromi* on *Bromus inermis* and *Septoria* sp. on *Poa pratensis* were isolated and grown in pure culture. Their pathogenicity was tested on the various grains and grasses.

This disease on quack grass (*Agropyron repens*) was first noted by Dr. J. J. Davis near Racine, Wisconsin, in 1892 (1). From material collected by him, Ellis and Everhart (4) described the fungus in 1893 as *Septoria agropyri*. The writer has noted this disease (in virulent form on quack grass) in various places in the vicinity of Madison, Wisconsin, during 1920 and 1921. In one case near the experiment station plots the disease was found on every plant, and on the average 90 per cent of the leaves were attacked and most of them were completely involved. In this particular patch of quack grass the number of heads developed was reduced from 50 to 60 per cent.

The symptoms of the disease are characteristic of Septoria infections. The leaves are severely attacked and become yellowish and soon turn brown and die. The pycnidia are somewhat smaller than the pycnidia of some other Septorias but they are prominent and seriatly arranged.

CAUSAL ORGANISM

As previously stated the causal fungus *S. agropyri* was described and named by Ellis and Everhart in 1893.

Mycelium.—Scant in culture, hyaline, septate, branched.

Pycnidia.—Subepidermal, black, subglobose, smooth, seriate, arranged, averaging 100 μ in diameter.

Ostiole.—Narrowly elliptical under stomata.

Pycnosporos.—(Fig. 7, D) Straight or curved, rounded ends, smooth, 3-septate, hyaline, slightly guttulate, 1.5–2.5 by 24–45 μ , most commonly 2 by 34 μ .

The fungus was readily obtained in pure culture by the employment of methods already described. The pycnosporos were germinated on potato-dextrose agar plates. Single germinating pycnosporos were then transferred to culture tubes.

Upon germination the pycnosporos enlarged somewhat and after from 6 to 8 hours, small buds were developed near the septa. These developed into conidia very similar to the pycnosporos. These conidia in turn germinated after 36 hours and thus colonies were formed, which were circular, raised, smooth, and of a pinkish rose color. After several days the surface became wrinkled and after two weeks it became black and more or less tough. Plants of *Agropyron repens* both out-of-doors and in pots in the greenhouse were inoculated which showed characteristic symptoms of the disease after two weeks. A large number of cereals and grasses were inoculated to determine the host range of this fungus. The data obtained are summarized in table 1 and the results represented diagrammatically in figure 3.

As shown in table 1 and figure 3, *S. agropyri* attacks *Agropyron repens* readily but none of the cereals or any of the other grasses tested. Other species of *Agropyron* were not available for testing at the time.

SUMMARY

1. The Septoria leaf-blotch of quack grass was found in Wisconsin by J. J. Davis in 1892. The causal fungus was first described in 1893 from Wisconsin material by Ellis and Everhart as *Septoria agropyri*.

2. As far as known the disease attacks only *Agropyron repens*. It is of no economic importance.

3. Mycelium, conidia, pycnidia, and pycnosporos of the fungus have been developed on potato-dextrose agar cultures.

4. The pycnosporos and conidia are hyaline, 3-septate. They most commonly measure 2 by 34 μ .

SEPTORIA LEAF BLOTCH OF BROME GRASS

This disease of brome grass (*Bromus inermis*) caused by *Septoria bromi* Sacc. was found by the writer on the experiment station grounds at

Madison, Wisconsin, June, 1920. It was not very abundant but since it was found along the borders of grain fields infected with Septoria diseases the fungus was isolated and inoculation experiments conducted on the various grains and some of the grasses. *Septoria bromi* was first found at Parma, Italy, by Saccardo, and described and named by him in 1884 (10). The fungus was first found in Wisconsin on *B. setarius* by E. T. Bartholomew in 1912 and in 1918 by J. J. Davis on *B. altissimus* (1, 2).

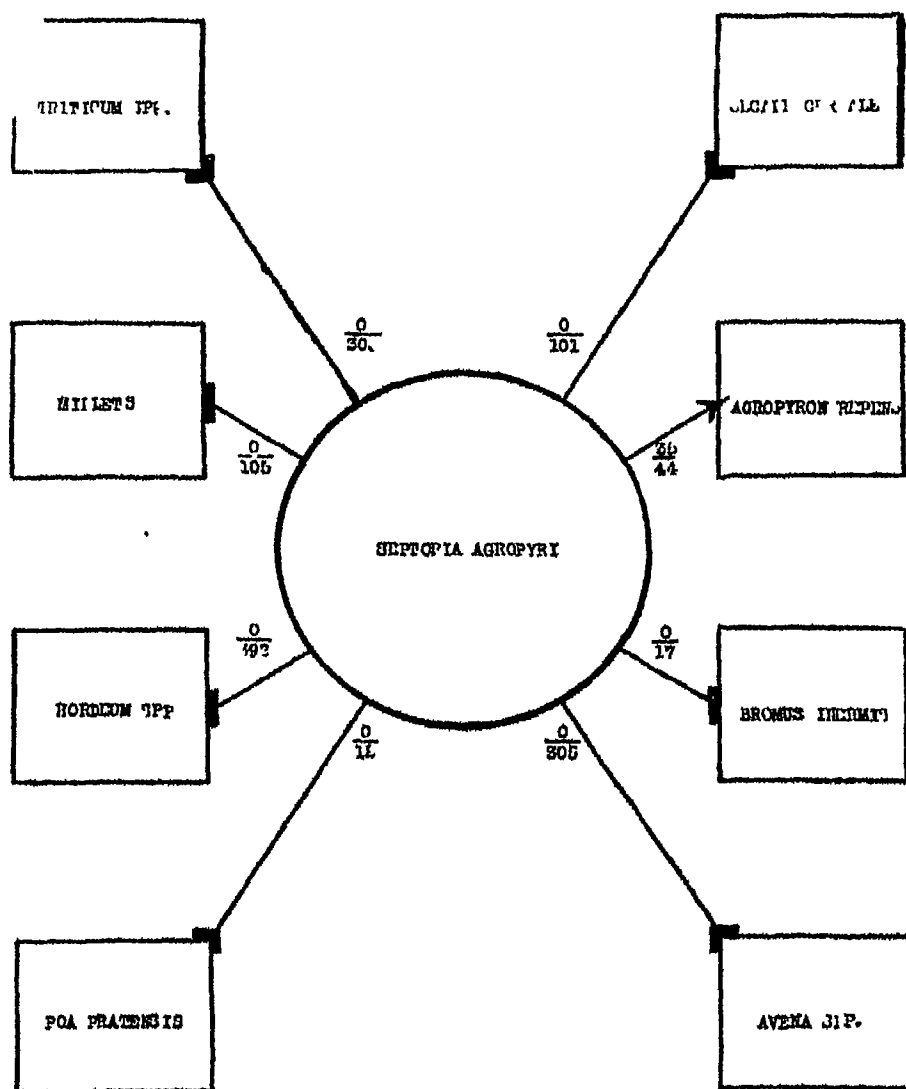


FIG. 3.—Diagram showing results of inoculation experiments with *Septoria agropyri*. Pycnospores were sprayed on the plants named in the rectangles. The denominator and numerator of the fraction show the number of leaves inoculated and the number of leaves infected respectively.

The symptoms of the disease on bromo grass (*B. inermis*) are very similar to those caused by Septorias on other graminaceous hosts. The

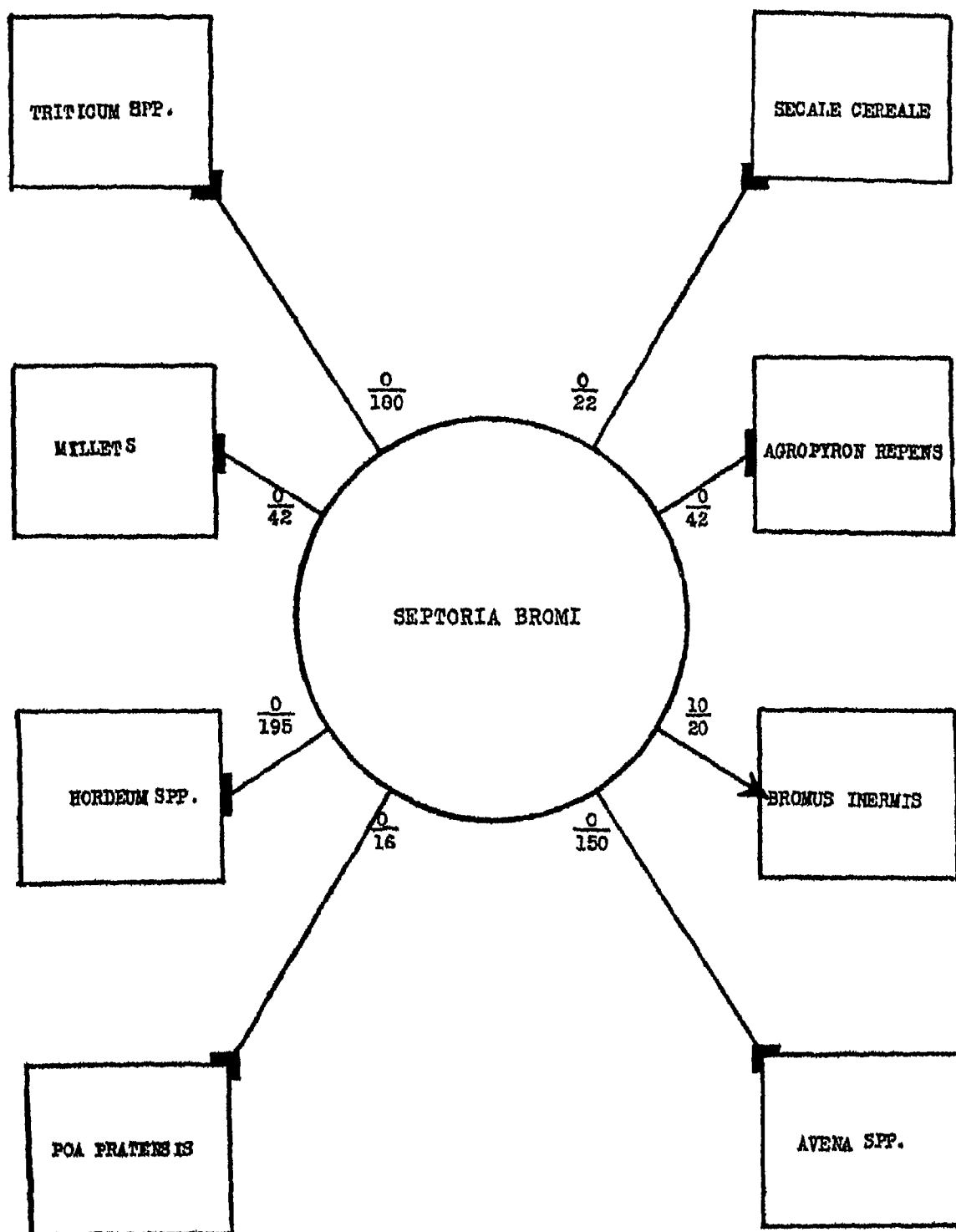


FIG. 4.—Diagram summarizing the results of inoculation experiments in which pycnosporos of *Septoria bromi* Sacc. were used as inoculum. The plants named in the rectangles were inoculated. The denominator and numerator of the fraction show the number of leaves inoculated and the number of leaves infected respectively. The arrow indicates infection.

infected leaves usually turn yellowish, dry up and die prematurely. The pycnidia are fully developed shortly before the leaf begins to dry up. The leaf blades and leaf sheaths only have been found attacked. The disease very seldom involves the whole leaf and consequently it is not considered to be of much economic importance. The fungus is readily isolated in culture. The mycelium is white in colonies but single hyphae are hyaline. The hyphae are branched, septate, and often decidedly vacuolate. The pycnidia are subepidermal, seriate, black, smooth, subglobose, 80-110 μ in diameter. The pycnosporos are (Fig. 7, B) cylindrical, septate, hyaline, usually curved, with ends rounded, slightly guttulate, measuring 1.5 to 2.5 by 31 to 57 μ usually 2 by 43 μ (Fig. 8). The pycnosporos obtained by soaking the leaf in water were sprayed with an atomizer on the leaves of a large number of species and varieties of cereals and grasses. The inoculated plants were covered for 48 hours immediately following inoculation. The results of these experiments are summarized in table 1 and are shown diagrammatically in figure 4.

As shown in table 1 and figure 4, *S. bromi* will attack only *Bromus inermis*. The host range on the genus *Bromus* has not as yet been worked out.

SUMMARY

1. The disease of bromic grass (*Bromus inermis*) caused by *S. bromi* has been previously reported only from Italy. It is reported here for the first time in the United States. Thus far, it has been found to be of negligible economic importance.
2. The fungus grows readily in artificial culture.
3. Pycnosporos measure 1.5 to 2.5 by 31 to 50 μ , mostly 2 by 43 μ .
4. The fungus will not infect the common cereals.

LEAF BLOTCH OF KENTUCKY BLUE GRASS

This disease of Kentucky blue grass (*Poa pratensis*), caused by *Septoria* sp., was found by the writer in September, 1920, on the campus of the University of Wisconsin at Madison. In certain areas every leaf was attacked by the fungus. It was found that this fungus compared favorably with the description of *Septoria graminum* given by Desmazieres (3). An examination of the type material showed that the lesions were long and narrow, decidedly delimited by the veins; also that the pycnidia which were almost invisible to the unaided eye were seriatly arranged, smaller, and measured 45 to 75 μ in diameter. The pycnosporos were found to measure 1 by 22 to 38 μ . In contrast to this the lesions on blue grass were not delimited by the veins. They

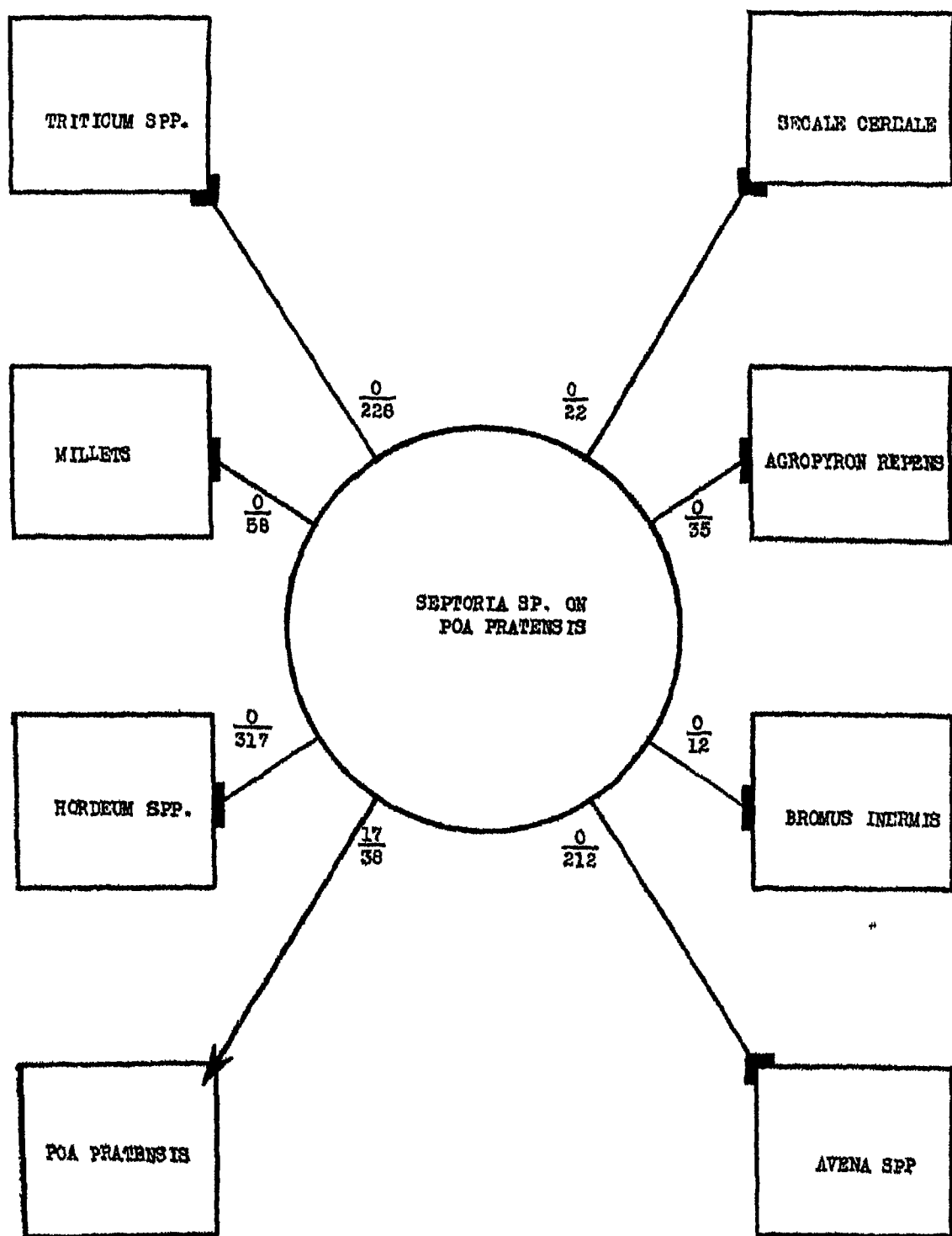


FIG. 5.—Diagram summarizing the results of inoculations with pycnosporos of *Septoria* sp. from *Poa pratensis*. Cereals and grasses inoculated are named in rectangles. The denominators and numerators of the fractions give the number of leaves inoculated and the number of leaves infected respectively. Arrows indicate infection.

formed a circular or oval spot either at one edge or across the whole leaf. The pycnidia, measuring 50 to 120 μ in diameter, were plainly visible to the unaided eye. They were much less closely seriate arranged. The pycnosporos measure 1 to 1.5 by 29 to 57 μ . From the comparison with the type material this disease on blue grass is distinct from *S. graminum* Desm. However, in a comparison of the description of *S. graminum* (which does not include measurements) the two organisms appear to be closely related. The organism on blue grass was cultured and the pycnosporos were used as inoculum in cross inoculation experiments to be described later. The symptoms were somewhat different from Septoria infections on the cereals. The lesions are more sharply delimited, the band of yellow narrower, and the center of the spot is killed quicker and finally becomes chocolate brown. The spots are dark rather than light colored at the time the pycnidia are mature which is not characteristic of Septoria diseases in the cereals. The infection is also more localized on the leaves than in the cereals. Entire leaves are seldom involved. The fungus grows readily in culture. On potato-dextrose agar the mycelium is scant, branched, hyaline, septate. The pycnidia are subepidermal, loosely seriate, smooth, black, subglobose, about 120 μ in diameter. The pycnosporos are (Fig. 7, C) cylindrical, straight or slightly curved, hyaline, 3-septate, septa often indistinct, and rounded at the ends.

The pycnosporos were obtained in pure culture and upon germination developed conidia and colonies. Growth in culture was found to be very similar to that of *S. tritici* and *S. agropyri*. Cross inoculations were conducted on different cereals and grasses, the results of which are given in table 1 and shown graphically in figure 5.

As shown in table 1 and figure 5, this *Septoria* sp. on *Poa pratensis* will not attack the cereals or certain grasses but attacks *Poa pratensis* fairly readily. The host range in the genus *Poa* has not been determined.

SUMMARY

1. The Septoria leaf blotch found on *Poa pratensis* at Madison, Wisconsin, is not *S. graminum* Desm.
2. The fungus grows well on potato-dextrose agar producing conidia abundantly.
3. Of the 40 different grains and grasses inoculated infections were obtained only on *Poa pratensis*.
4. The pycnosporos of this fungus measure 1 to 1.7 by 30 to 50 μ , mostly 1.5 by 30 μ .

TABLE 1

Summary of results from inoculating various grains and grasses with seven species of *Septoria* and *Leptosphaeria avenaria*.

Hosts	Sources of inoculum ¹	No. of leaves		Hosts	Sources of inoculum ¹	No. of leaves	
		Inoculated	Diseased			Inoculated	Diseased
<i>Avena barbata</i>	1	41	9	<i>Avena brevis</i>	1	44	4
	2	29	0		2	22	0
	3	19	0		3	22	0
	4	109	0		4	136	0
	5	131	0		5	127	0
	6	29	0		6	36	0
	7	181	0		7	168	0
	8	25	0		8	14	0
<i>Avena fatua</i>	1	43	7	<i>Avena nuda chinensis</i>	1	36	4
	2	31	0		2	52	0
	3	8	0		3	20	0
	4	171	0		4	108	0
	5	112	0		5	136	0
	6	28	0		6	23	0
	7	170	0		7	185	0
	8	12	0		8	26	0
<i>Avena sativa</i> (Culbertson)	1	96	18	<i>Avena sativa nigra</i>	1	40	11
	2	38	0		2	14	0
	3	18	0		3	19	0
	4	170	0		4	133	0
	5	149	0		5	126	0
	6	26	0		6	12	0
	7	176	0		7	184	0
	8	21	0		8	29	0
<i>Avena sativa orientalis</i>	1	31	5	<i>Avena sterilis</i> (b)	1	43	8
	2	23	0		2	52	0
	3	7	0		3	17	0
	4	119	0		4	137	0
	5	124	0		5	115	0
	6	9	0		6	20	0
	7	167	0		7	105	0
	8	22	0		8	21	0

¹ The inoculum used in these experiments consisted of spore suspensions of the following fungi:

1. *Leptosphaeria avenaria*
2. *Septoria agropyri*
3. *Septoria bromi*
4. *Septoria nodorum*

5. *Septoria passerinii*
6. *Septoria secalis*
7. *Septoria tritici*
8. *Septoria* sp. on *Poa pratensis*

Hosts	Sources of in- oculum	No. of leaves		Hosts	Sources of in- oculum	No. of leaves	
		Inoc- ulated	Dis- eased			Inoc- ulated	Dis- eased
<i>Avena sterilis</i> (a)	1	56	11	<i>Avena strigosa</i>	1	47	3
	2	12	0		2	38	0
	3	4	0		3	18	0
	4	130	0		4	136	0
	5	129	0		5	136	0
	6	7	0		6	26	0
	7	211	0		7	183	0
	8	17	0		8	25	0
<i>Hordeum de- ficiens</i>	1	31	0	<i>Hordeum hor- fordianum</i>	1	77	0
	2	26	0		2	51	0
	3	27	0		3	20	0
	4	132	0		4	287	0
	5	129	91		5	287	224
	6	32	0		6	37	0
	7	188	0		7	406	0
	8	38	0		8	41	0
<i>Hordeum disti- chon erectum</i>	1	18	0	<i>Hordeum disti- chon nudum</i>	1	24	0
	2	32	0		2	27	0
	3	6	0		3	21	0
	4	140	0		4	130	0
	5	129	110		5	159	135
	6	29	0		6	26	0
	7	215	0		7	200	0
	8	28	0		8	26	0
<i>Hordeum disti- chon nutans</i>	1	19	0	<i>Hordeum jubatum</i>	1	18	0
	2	31	0		2	11	0
	3	17	0		3	12	0
	4	172	0		4	0	0
	5	165	130		5	0	0
	6	30	0		6	0	0
	7	193	0		7	91	0
	8	15	0		8	0	0
<i>Hordeum vulgare coerulescens</i>	1	22	0	<i>Hordeum vul- gare hexas- trichum</i>	1	20	0
	2	25	0		2	32	0
	3	10	0		3	17	0
	4	111	0		4	136	0
	5	149	124		5	128	98
	6	23	0		6	16	0
	7	204	0		7	186	0
	8	16	0		8	17	0

Hosts	Sources of inoculum	No. of leaves		Hosts	Sources of inoculum	No. of leaves	
		Inoculated	Diseased			Inoculated	Diseased
Hordeum vulgare himalaya	1	21	0	Hordeum vulgare nigrum	1	23	0
	2	30	0		2	31	0
	3	25	0		3	6	0
	4	192	0		4	135	0
	5	131	101		5	139	91
	6	35	0		6	22	0
	7	184	0		7	321	0
	8	31	0		8	37	0
Hordeum vulgare pallidum	1	20	0	Hordeum vulgare trifurcatum	1	25	0
	2	38	0		2	47	0
	3	23	0		3	8	0
	4	150	133		4	129	0
	5	150	0		5	149	110
	6	11	0		6	43	0
	7	239	0		7	210	0
	8	17	0		8	18	0
Barley, Mauriat X Lion	1	0	0	Secale cereale	1	29	0
	2	11	0		2	101	0
	3	0	0		3	22	0
	4	136	0		4	345	232
	5	274	214		5	183	0
	6	21	0		6	72	71
	7	123	0		7	256	138
	8	31	0		8	32	0
Triticum aestivum	1	118	0	Triticum compactum	1	32	0
	2	101	0		2	30	0
	3	74	0		3	11	0
	4	769	534		4	100	121
	5	590	0		5	148	0
	6	90	0		6	28	0
	7	753	550		7	191	104
	8	66	0		8	31	0
Triticum dicoccum	1	56	0	Triticum durum	1	35	0
	2	55	0		2	25	0
	3	24	0		3	21	0
	4	317	218		4	160	122
	5	319	0		5	148	0
	6	53	0		6	20	0
	7	411	0		7	181	124
	8	36	0		8	33	0

Hosts	Sources of in- oculum	No. of leaves		Hosts	Sources of in- oculum	No. of leaves	
		Inoc- ulated	Dis- eased			Inoc- ulated	Dis- eased
<i>Triticum mono- coecum</i>	1	32	0	<i>Triticum polonicum</i>	1	31	0
	2	18	0		2	30	0
	3	8	0		3	16	0
	4	160	109		4	172	120
	5	132	0		5	160	0
	6	18	0		6	24	0
	7	209	134		7	203	124
	8	9	0		8	22	0
<i>Triticum spelta</i>	1	26	0	<i>Triticum turgidum</i>	1	26	0
	2	30	0		2	14	0
	3	18	0		3	8	0
	4	216	166		4	159	115
	5	152	0		5	122	0
	6	39	0		6	30	0
	7	138	109		7	183	133
	8	19	0		8	12	0
<i>Agropyron repens</i>	1	35	0	<i>Bromus inermis</i>	1	24	0
	2	41	38		2	17	0
	3	14	0		3	20	10
	4	234	0		4	23	0
	5	145	0		5	11	0
	6	34	0		6	14	0
	7	216	0		7	110	0
	8	35	0		8	12	0
<i>Chaetochloa italica</i>	1	17	0	<i>Chaetochloa viridis</i>	1	19	0
	2	31	0		2	16	0
	3	6	0		3	17	0
	4	61	0		4	109	0
	5	80	0		5	102	0
	6	15	0		6	20	0
	7	148	0		7	19	0
	8	16	0		8	11	0
<i>Echinochloa crus-galli</i>	1	17	0	<i>Panicum clandestinum</i>	1	25	0
	2	21	0		2	9	0
	3	10	0		3	0	0
	4	109	0		4	96	0
	5	78	0		5	71	0
	6	29	0		6	24	0
	7	178	0		7	127	0
	8	0	0		8	13	0

Hosts	Sources of inoculum	No. of leaves		Hosts	Sources of inoculum	No. of leaves	
		Inoculated	Diseased			Inoculated	Diseased
<i>Pennisetum glaucum</i>	1	15	0	<i>Poa pratensis</i>	1	54	0
	2	25	0		2	12	0
	3	9	0		3	16	0
	4	76	0		4	237	96
	5	82	0		5	206	0
	6	22	0		6	25	0
	7	151	0		7	183	80
	8	10	0		8	38	17

Table 1 has been discussed more or less in the several parts of this paper under the different diseases. This table contains data from all of the inoculation experiments conducted. Eight distinct species of *Septoria* have been used separately as inocula in inoculating each of the plants given in this table. The data show that *Leptosphaeria avenaria* from oats, *Septoria passerinii* from barley, *S. secalis* from rye, *S. agropyri* from quack grass, *S. bromus* from brome grass, and *Septoria sp.* from *Poa pratensis* are sharply specialized to the hosts on which each was found. While *S. tritici* and *S. nodorum* are less specialized in that, in both cases, they attack rye and *Poa pratensis* in addition to the different species and varieties of wheat.

SUMMARY

1. On oats, wheat, barley, rye, and certain grasses (Fig. 6) there is a group of similar diseases caused by a number of closely related species of *Septoria*. These species have been found to be morphologically and physiologically distinct. The ascigerous stage of one of these species has been found.

2. Certain of these diseases occur widely distributed throughout the world and may become of considerable economic importance, while others are known to occur only locally and, as far as known, are of no economic importance.

3. Most of these diseases occur only on the leaves. One of them, however, is found on the glumes of wheat. In this case the lesions are localized, usually brown in color with pycnidia present in the later stages of development. On the leaves the lesions are, in general, similar on the various hosts. In each case, the lesions are localized, of various

sizes and forms, variously bleached or discolored, and in the later stages they show the presence of dark colored pycnidia.

4. On oats there occurs only one disease of this group. It is caused by the fungus previously known as *Septoria avenae* Frank. However, the ascigerous stage of this fungus has been found which proves to be

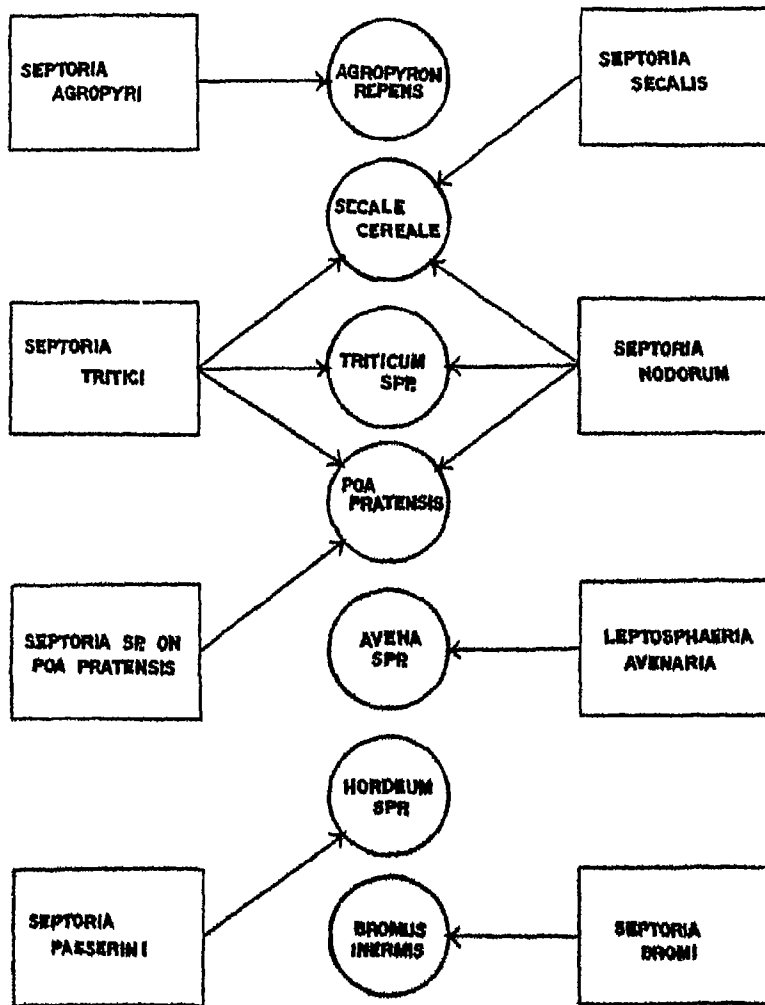


FIG. 6. --Summary of the positive results obtained as shown in table 6. The circles represent the plants inoculated; the rectangles, the organisms with which the inoculations were made. Arrows indicate infection.

a species of *Leptosphaeria*. The specific name *avenae* having been previously used in the genus *Leptosphaeria* for another fungus, a new species name *L. avenaria* is given. This fungus attacks only oats. On wheat there are two diseases caused by *Septoria nodorum* Berk. and *S. tritici* Desm., respectively. Both of these fungi have the same host range. They both attack rye and Kentucky blue grass in addition to wheat.

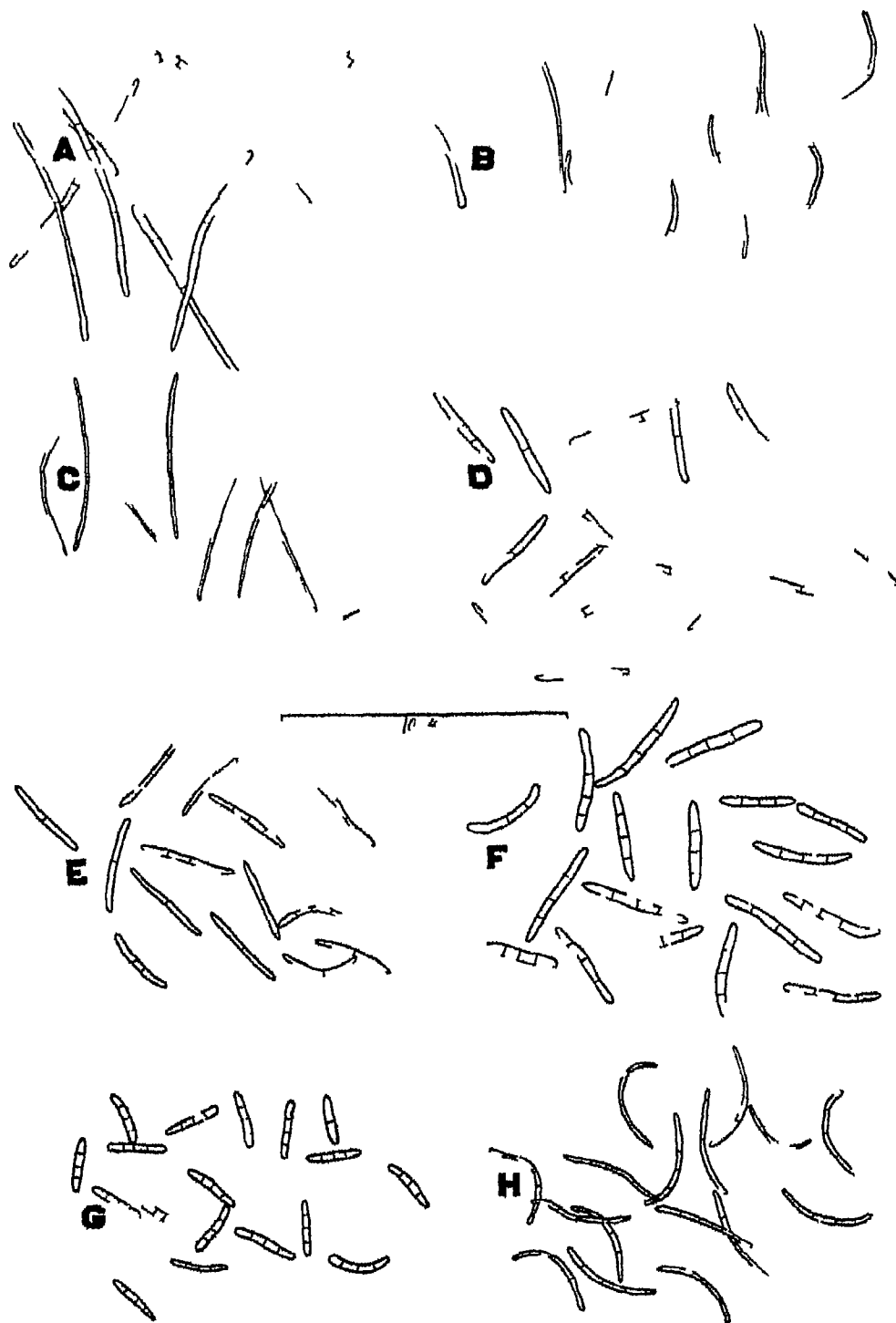


FIG. 7.—Camera lucida drawings of pycnospores collected in the field. Magnification the same throughout. A *Septoria tritici* on wheat. B *Septoria bromi* on brome grass (*Bromus inermis*). C *Septoria* sp. on Kentucky blue grass (*Poa pratensis*). D *Septoria agropyri* on quack grass (*Agropyron repens*). E *Septoria secalis* on rye. F *Leptosphaeria avenaria* on oats. G *Septoria nodorum* on wheat. H *Septoria passerinii* on barley.

There is also a disease of rye caused by *S. secalis* Prill. and Del., which is parasitic on rye only.

On barley there is only one disease of this group which is caused by *S. passerinii* Sacc. A disease of quack grass, brome grass, and Kentucky blue grass, caused by *S. agropyri* Ell. and Ev., *S. bromi* Sacc., and *Septoria* sp., respectively.

5. While the eight species of fungi concerned are in general very similar, they are also distinctly different both morphologically and

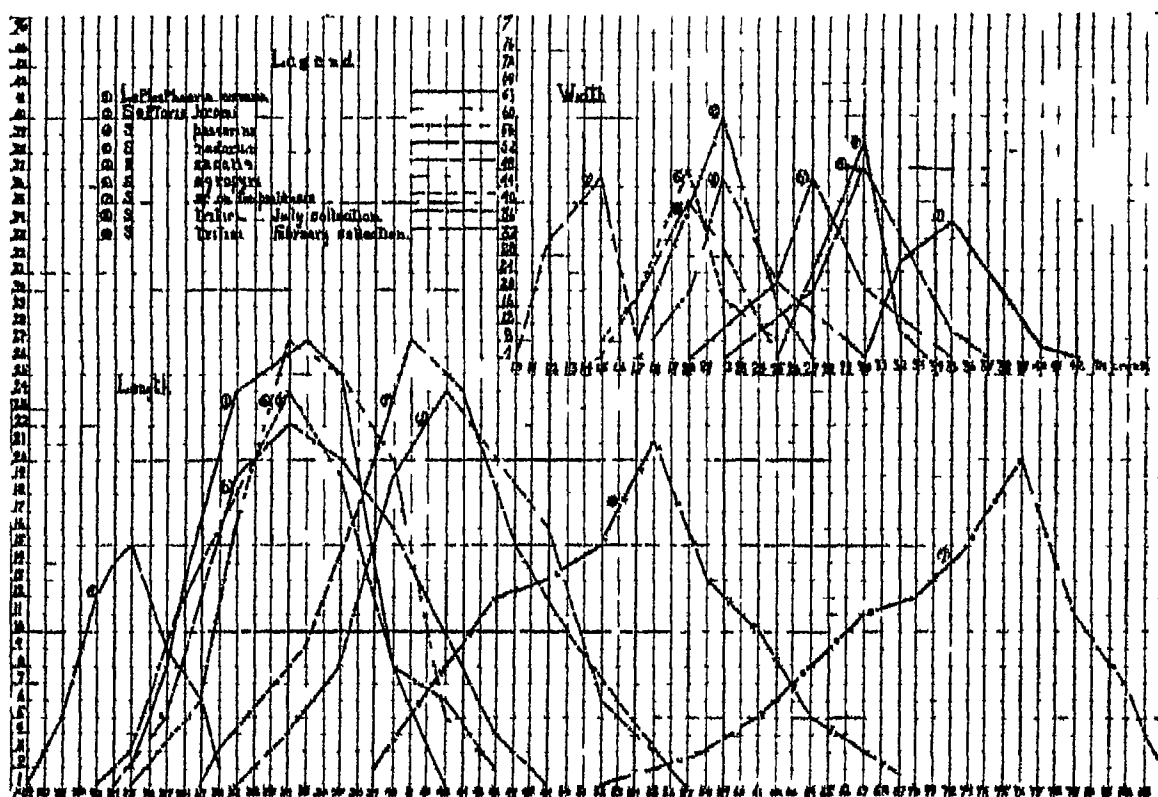


FIG. 8.—Graph showing widths and lengths of pycnospores of different fungi as indicated. Each graph represents 100 spores measured. In each case the ordinates indicate the percentages of spores of different length (in microns) and width in tenths of microns, as indicated by the abscissae.

physiologically. The ascigerous stage has been definitely found for only one of these fungi, namely *Leptosphaeria avenaria*. The pycnidia of each, however, are fairly characteristic both as to form and size, as shown in figures 7 and 8. *Septoria tritici* has the largest pycnospores and *S. nodorum* the smallest. The pycnospores of *Septoria* sp. from *Poa pratensis* are the most slender. Those of *S. bromi* and *S. passerinii* are the most alike, those of the latter, however, being somewhat shorter. The pycnospores of *Leptosphaeria avenaria* and *S. agropyri* are similar in

general shape those of the former being distinctly wider. All eight species grow readily and produce pycnidia on artificial media. *Septoria tritici*, *S. passerinii*, *S. agropyri* and *Septoria* sp. from *Poa pratensis*.

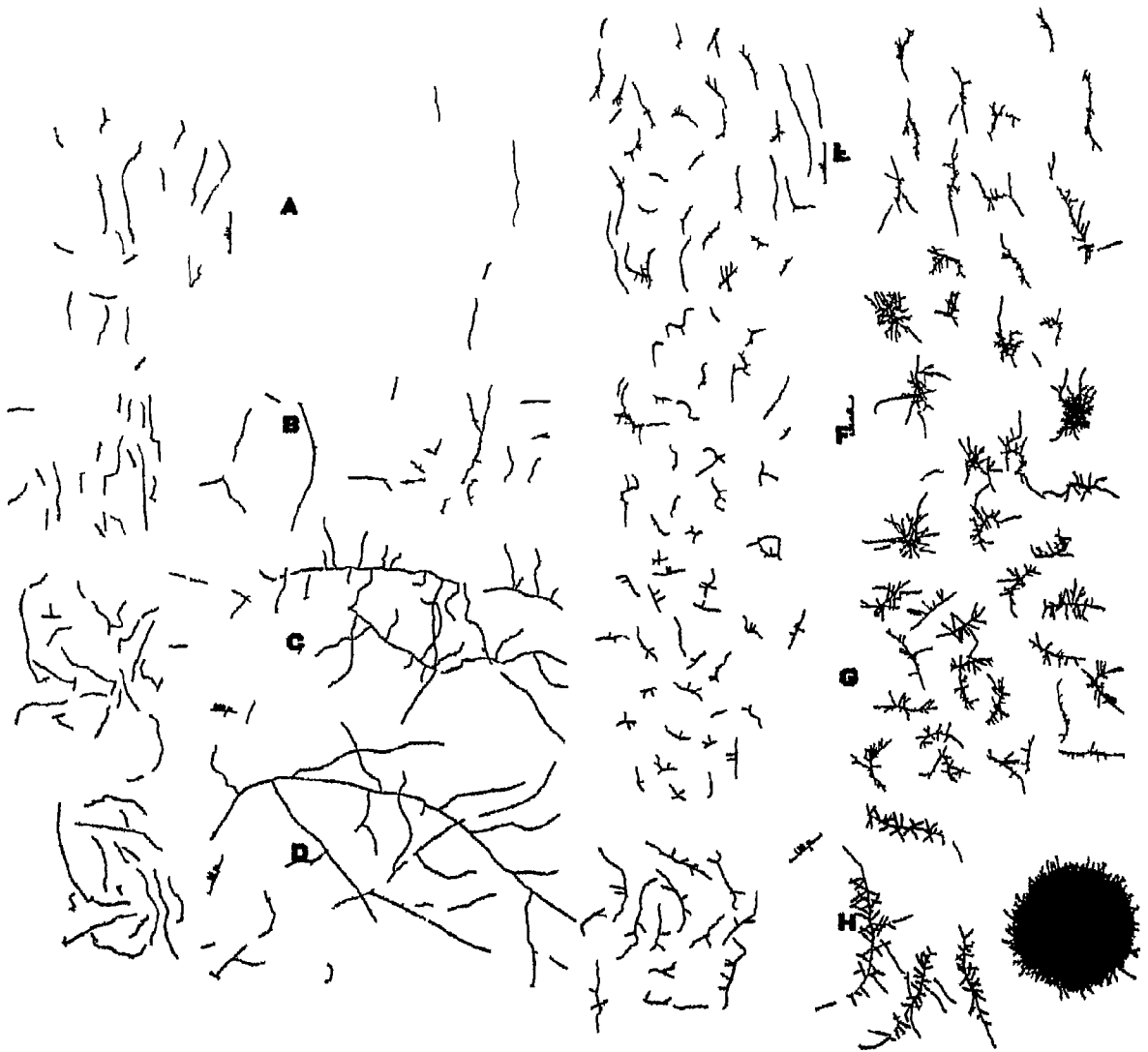


FIG. 9.—Camera lucida drawings of pycnosporos germinating on potato dextrose agar plates. A. *Leptosphaeria avenaria*. B. *Septoria bromi*. C. *S. secalis*. D. *S. nodorum*. E. *S.* sp. on *Poa pratensis*. F. *S. passerinii*. G. *S. agropyri*. H. *S. tritici* also 5 day old colony.

With the exception of *S. tritici* (H) growth at left and right is 24 and 48 hours old respectively. Growth of *S. tritici* (H) at left is 24 and at center 48 hours old. At right is a 5 days old colony.

produce conidia from scant hyaline mycelium. As cultures get older olivaceous hyphae are produced. In contrast to these, *Leptosphaeria avenaria*, *Septoria nodorum*, *S. secalis*, and *S. bromi* produce abundant mycelium, which is cottony at first, later becoming more or less olivaceous.

ous. In the latter group conidia have been noted only in cultures of *S. nodorum*, and there only rarely. In the latter group the pycnosporos germinate by the production of an abundant mycelium, while in the former only scant mycelium and abundant conidia are formed (Fig. 9).

6. The pathological changes in the host tissue caused by these eight fungi are similar. The mycelium grows between the cells of the parenchyma. The pycnidia are subepidermal in substomatal chambers. In the cases of *Leptosphaeria avenaria* on oats, *S. tritici* and *S. nodorum* on wheat, and *S. passerinii* on barley it was found that the germ tubes penetrate the cuticle. They then grow in between the epidermal and parenchyma cells.

7. As a result of inoculation experiments the incubation period has been found to be from 11 to 15 days. The incubation period is here considered to be the time elapsed between the sowing of the spores on the host tissue and the maturing of the pycnidia. The first symptoms become noticeable about 8 days after inoculation.

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THE TRANSMISSION OF SUGAR-CANE MOSAIC BY *APIIS MAIDIS* UNDER FIELD CONDITIONS IN PORTO RICO.

C. E. CHARDON AND R. A. VINE.

WITH ONE FIGURE IN THE TEXT.

During the course of an investigation on the ecological factors bearing on the spread in the field of the mosaic disease of sugar-cane, the senior author has repeatedly realized the importance of insects as the probable agents for the transmission of the disease. The relation of rainfall, humidity, temperature and age of the cane plants to the spread of the disease is considered as important, especially since very little is known about them; but the question of the transmission of the disease by insects has come forth as vital, since it is only after a thorough comprehension of it, that we can understand how the spread of the disease takes place. How is the disease transmitted in the field? What insects are responsible for its transmission in the field? These questions have, to the present time, defied all the attempts made by entomologists to solve them.

The work of Brandes (1), in this respect, has proven to be very guiding and suggestive. He succeeded in transmitting the disease from diseased to healthy plants by means of the corn aphid, *Aphis maidis*, in the greenhouses at Washington, D. C. His results have been accepted as conclusive. Brandes, however, did not actually solve the problem of insect transmission in the field. In the discussion of his results he says (p. 137): "Just what insects are responsible for dissemination in the cane regions remains to be proved." The fact that *Aphis maidis* does not ordinarily occur on cane has led entomologists to pay little or no attention to it, and has induced them to try extensive cage experiments using other insects which are known to occur on sugar-cane. All these attempts have met with failure.

Smyth (5), in 187 trials with different insects, had only 4 successful takes, and each one of them with a different species of insect. He says (p. 111): "In view of the small number of successful inoculations secured, as compared with the rather large number of tests made, under conditions which were considered favorable, the question of insect transmission of cane mottling disease can not be looked upon as settled." In another publication (6), he states that out of 310 additional experiments, only 9 gave positive results. The work of Smyth has been con-

tinued by Wolcott, who has published a comprehensive study (7) of all the minor sugar-cane insects of Porto Rico. The importance of these minor insects, he says (p. 5): "lies not in the injuries which they cause directly, but in the possible connection that may exist between one, or some of them, and the rapid spread of a disease, new to Porto Rico, the mottling or mosaic disease of sugar-cane." He paid special attention to the study of the leafhoppers, whose abundance in sugar-cane fields has attracted the attention of all workers on the disease. Wolcott accepts Brandes' work, but in regard to the occurrence of *Aphis maidis*, he says (p. 34): "The corn aphid has not been found on cane in Porto Rico. It can not in any way be considered responsible for the transmission of mosaic disease of sugar-cane under field conditions in Porto Rico." Further transmission experiments carried on by Wolcott, at the Insular Experiment Station, and using the common leafhopper *Kolla similis*, have met with failure. The results of the insect transmission experiments at the Federal Experiment Station at Mayaguez, P. R., have not been published yet, but 86 trials have been made by the entomologist, especially with the yellow aphid, *Sipha flava*, and all of them have given negative results. Lodeboer (4), in a short report from Java, reports a successful inoculation with *Aphis sacchari*, but he does not mention the number of failures, and consequently, his work seems questionable. Thus the situation as regards the transmission of the disease by insects, and specially, the spread of the disease in the field remains, as Prof. F. S. Earle (3, p. 18) says: "dependent on some factor or factors as yet absolutely undiscovered."

In a very recent publication, Bruner (2) has succeeded in duplicating Brandes' results with *Aphis maidis*, at the Experiment Station at Santiago de las Vegas, Cuba. In two series of experiments, he succeeded in getting 14.2 and 11.1 per cent positive inoculations with the use of the corn aphid. In his opinion, however, the question of the insect transmission in the field is still unsolved. He says (p. 21): "Even if the evidence shows that the green corn aphid can transmit the mosaic disease of sugar-cane, it does not solve the problem of dissemination, because this insect does not attack cane under ordinary field conditions" (in translation).

In view of the diversity of opinion of different authorities on this question, we have conducted field experiments, which in our opinion, have given us a clue towards its solution. We are attempting, for the first time, to give an explanation of how the spread of the disease in the field actually takes place. The sequence of events leading us to these experiments are as follows:

In one of the fields under observation by the senior author, located at Santa Rita, P. R., Mr. Wolcott found an abundance of *Aphis maidis* in the central whorl of leaves of the young cane plants. He could not say where the aphids came from, there being no corn nor sorghum in the immediate vicinity, but it was observed that this unexpected invasion by the aphids was followed by a sudden spread of the disease. The exact percentage of mosaic infection in this field follows:

<i>Field No. 17:</i>	Oct. 11	1 1 per cent		
Santa Rita.	Nov. 12	11 3 " "	(aphids collected Oct. 26)	
	Dec. 9	35 8 " "		
	Jan. 7	43 2 " "		
	Feb 20	51 4 " "		

A due importance was attached to this phenomenon, and in subsequent trips around the island, the senior author made special search for the aphid. It was found to occur in practically every sugar-cane field in Porto Rico. It was not living on cane but occurred on various common grasses. Curiously, however, after the weeding of a field, the aphids passed to the cane plants and lived in the central whorl of leaves. This obligate change of hosts, that is, from the grasses to the cane, was invariably followed by a sudden spread of the disease, irrespective of the presence or absence of leafhoppers or other minor insects.

With these facts on hand, highly suggestive of success, the need of experimentation on the subject became urgent. The Fajardo Sugar Co. very kindly volunteered its services to construct the cages needed for the experiment. We are specially indebted to the manager, Mr. Jorge Bird, for the interest he has shown in our work. The experiment was started under the direction of the junior author, in charge of the Fajardo Sugar Co. Experiments Station, and his immediate assistant Mr. Luis de Colis, who has taken special pains in the details of the experiment and which have decidedly contributed towards its final success.

Two insect-proof cages, 24 by 14 by 5 feet were constructed, using a fine mesh cheese-cloth. They were erected in the middle of a cane field, so as to be under perfect field conditions (Fig. 1), 48 seeds of the susceptible variety DIII were planted in each cage at the ordinary field distances. In one case half of the seed used was diseased and the other half healthy. Field observations in the adjoining fields had shown *Aphis maidis* to occur on the common weed "malojillo" (*Eriochloa subglabra*). An abundance of this weed was planted in cage A, and a number of corn aphids introduced in it. Cage B was kept free from weeds and insects, so as to serve as a check on cage A.

A perfect germination of the cane seeds was secured, and the "mal-

jillo together with other weeds grew luxuriantly in cage A. All weeds were systematically kept down in cage B. One month after planting the seed when the cane plants were 6-8 inches high a thorough weeding of cage A was done by a laborer. Among the weeds killed were *Imo-*
chloa subglabra *Cyperus rotundus* *Echinochloa colona* *Eleusine indica*
Syntherisma sanguinalis *Portulacca cleracea* *Chamaecyprip hypericifolia*
Commelina sp. *Amarantus spinosus* and *Ipomoea tiliacea*. Among them
 the milojillo (*Imoichloa subglabra*) was decidedly the preferred host



FIG. 1. A view of the field in which the following experiment was conducted at Tajiri, P. R.

for the aphid, although a few were seen on *Echinochloa*, *Eleusine* and *Syntherisma*.

The weeding was done at 5 P. M. Early in the next morning the aphids were seen apparently feeding in the central whorl of leaves of the young cane plants. They stayed on the cane for some time but as soon as weeds started to come up they were seen on their favorite hosts.

The results followed in quick succession. Two weeks later an evident case of secondary infection appeared in cage A. 17 days later 4 more cases appeared, and at the end of two months 15 out of the 24 plants had contracted the disease. This represents an infection of 62.5 per cent.

In cage B, used as a check, no cases of transmission have occurred and all the healthy plants have remained healthy for a period of over three months.

Three of the grasses present in cage A, *Syntherisma sanguinalis*, *Eleusine indica* and *Echinochloa colona*, very unexpectedly came out showing true symptoms of mosaic. It will be recalled that all three of them acted as hosts for the aphids. *Eriochloa subglabra*, the favorite host for the insect, did not show symptoms of mosaic, being apparently immune to it. This is not the first record of grasses becoming affected with sugar-cane mosaic. As a result of experiments Brandes reports three species which became affected with the disease. One of them, *Syntherisma sanguinalis*, is the same one that we report here.

It must be stated here to the credit of the experiment, that the region of Fajardo is far remote from all possible source of infection. There were no cases of the disease in all the fields adjoining our experiment. Consequently, the cases of secondary infections in cage A, must have come from the disease plants present within the cage itself. The disease must have also been transmitted by *Aphis maidis*, the only insect present. The proof for this last statement is that in cage B, where the aphid was absent, no transmission of the disease took place.

No further discussion nor comment about the experiment needs to be made, but its importance, in the opinion of the authors can not be overestimated. It opens a field of investigation partially neglected before, and suggests many experiments of an entomologic and agronomic nature bearing on the practical control of the disease. We consider it as a positive duplication of Brandes work, under ordinary field conditions at Fajardo, P. R., and with the use of insects common to that region. Since the rainfall, humidity and temperature records at Fajardo, during the time the experiment was running do not differ substantially from those of the rest of the island, we are led to believe that they represent the field conditions of most of the sugar-cane growing regions of Porto Rico.

We do not feel justified yet, to draw definite conclusions with regard to the insect transmission of the mosaic disease of sugar-cane and our experiments are given here merely as an introduction to the problem of insect transmission in the field. Much remains to be done yet. We feel safe, however, in saying that we have field observations and experimental evidence pointing out to the facts that:

1. *Aphis maidis* is found, with more or less abundance, in various grasses occurring in the sugar-cane fields of Porto Rico.

2. After the weeding of the fields, *Aphis maidis* passes to the sugar-cane plants living in the central whorl of leaves for a period of time.

3. During the short time that it stays on cane, *Aphis maidis* transmits the infective substance of the sugar-cane mosaic and carries the disease from diseased to healthy plants.

RIO PIEDRAS, PORTO RICO

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A METHOD OF PHOTOGRAPHING SPORE DISCHARGE FROM APOTHECIA

F. DICKSON AND W. R. FISHER

WITH TWO FIGURES IN THE PLATE

During some studies on the factors governing apothecial formation in *Sclerotinia libertiana* Pekl., an exceptionally large number of apothecia were obtained, in several instances more than one hundred of them arising from individual sclerotia. It was suggested by Professor H. H. Whetzel that an attempt be made to procure a photograph of the spore-cloud emanating from these fruit bodies, and, after some experimenting, the endeavour met with unusual success. This being the case, it is thought worth while to outline the method employed in making the photograph, in order that others may profit by our experiences. Without doubt, such illustrations may be effectively employed to give a vivid idea of the aerial dissemination of fungous spores.

So far as can be ascertained by the writer, no such attempt at photographing spore discharge has ever been reported before, although Pethybridge,¹ depicts what he calls "cloud-like puffs of spores" from apothecia of *Sclerotinia sclerotiorum* from potato. In this case, however, it is surmised that the apothecia were allowed to discharge their spores upon a flat surface and were then photographed, the spores not being actually in motion at the time.

The photographs accompanying this note were made by the junior author, photographer for the Department of Plant Pathology, using a camera equipped with a Voightlaender "dynar lens." An Eastman "portrait super-speed film," which is probably the fastest on the market at the present time, was used. The aperture was set at f. 6. 3 and the exposure given was 1/100th of a second.

The essentials upon which success depends may be summarized as follows:—

1. The apothecia, apart from being sufficiently abundant, must be in good condition, in order to produce a cloud of maximum density.
2. The background must be black or as nearly black as possible.

The matter of the actual growing of the apothecia will not be dealt with here but will be reserved for a future paper. In order to ensure that they would be in good condition for discharging spores a layer of absorbent cotton, saturated with water, was first placed on the inside of

¹ Pethybridge, George H. Potato diseases in Ireland. Jour. Dept. Agric. and Tech. Instruct. Ireland 10. 241-250. 8 fig. 1910.

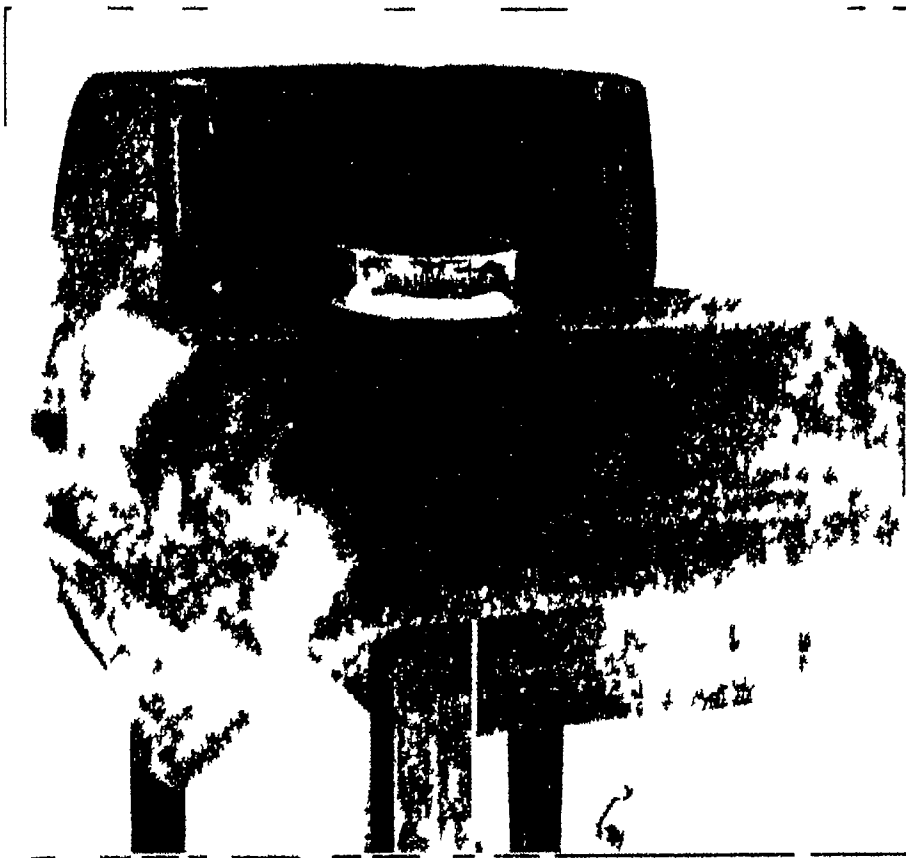


Fig 1 Photograph showing the position of the dish containing apothecia, and the background when ready for photographing



Fig 2 Photograph showing spore cloud emanating from apothecia of *Sclerotinia libertiana* Fekl.

the lid of a six inch "stender dish." The sclerotia with apothecia were then arranged upon this layer of cotton in the exact position in which they were to be photographed, and the stender dish itself inverted over them. The whole was then placed out-of doors in a shaded spot for three days to allow the ascospores to mature. With this arrangement, no disturbance of the apothecia was necessary before the photograph was actually taken. This was important, for, as is well known, discharge of ascospores from the apothecia takes place on the least disturbance, especially if the cover should be removed so as to allow slight air currents to reach the apothecia. About two hours before the photograph was taken, the dish containing the apothecia was taken inside and placed in the position shown in figure 1, which illustrates the general plan adopted.

For a background, an empty box was first lined on the inside with black paper to render it light proof, and this, in turn, was covered with black velvet. The table was then arranged in a corridor, near a doorway, so that direct sunlight came from one side, illuminating the apothecia without shining into the box. In this way, a jet-black background was obtained.

At first, some difficulty was experienced in the focusing, for this, of necessity, had to be done while the apothecia were still covered. To overcome this difficulty, a small object (a "thumb-tack" was used) was placed on the top of the dish, immediately over the centre of the mass of apothecia and a sharp focus on this obtained. The final results showed that this gave a perfect focus on the apothecia when the cover was removed. Screens were so arranged as to prevent the wind from dispersing the spore-cloud.

When everything was prepared, the photographer stood ready at the bulb of the camera and, at a given word, the stender dish was quickly removed from the apothecia. Nothing happened for about one second, and then a sudden cloud of spores was discharged into the air, the exposure being made at the proper instant with the result shown in figure 2.

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**ABSTRACTS OF PAPERS PRESENTED AT THE FOURTEENTH
ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL
SOCIETY, BOSTON, MASSACHUSETTS, DECEMBER 27
TO 30, 1922.**

Recent studies on control methods of Texas root rot. J. J. TAUBENHAUS AND D. T. KILLOUGH.

Statements abound to the fact that the Texas root rot disease may be controlled by deep plowing or crop rotation in which non-susceptible hosts are grown during the rotation period. In our studies, we have found that these recommendations were not always successful because they were not made on a knowledge of the life history of the causal organism. The causal organism winters over on living roots of susceptible hosts.

Deep fall plowing does not control root rot because such a practice does not necessarily kill the cotton roots; neither does it always destroy the susceptible weeds, especially the Small Flowered Pink Morning Glory, *Ipomoea trichocarpa*, which is so common in the black lands of Texas.

Likewise, crop rotation does not control root rot even though a three- or four-year system is adopted in which non-susceptible crops are grown. In this case, the failure at control is due to the fact that the causal organism is permitted to live over during the fall and winter months on roots of perennial weeds which are commonly found on the stubble land of the grain or cereal crops which are used in the system of rotation.

It is possible to control root rot by fall plowing or crop rotation provided clean culture is practiced not only during the growing season, but also during the fall and winter months.

Cartoons in Agricultural Teaching. R. R. REPPERT.

The paper deals with the use of cartoons in teaching agricultural truths. During the past few months, cartoons, depicting the life history of the boll weevil, especially those phases having close relationship to fall and winter control measures, have been put out by the Division of Extension Entomology and Plant Pathology of the A. and M. College of Texas. The high interest they have aroused among the farmers over the state indicates they are a valuable means of extending knowledge on entomological subjects. It is believed that added interest may in the same way be given to subjects of plant diseases, among practical farmers with insufficient technical training to fully appreciate the more scientific phases of the subject.

The growth reactions of certain fungi to their staling products. C. BOYLE.

Fungi which develop for some time on solid or liquid media sooner or later show a marked diminution in rate of growth, varying with the organism and the medium used. Such "staling" effect is characteristic of a species of *Fusarium* originally isolated from

decaying apples when grown on Richard's solution, apple or potato extracts. The progress of staling was measured by the effect of the staled liquid on germination and growth of spores of *Botrytis cinerea*.

Growth of *Fusarium* sp. in the three media mentioned caused a change in pH towards the alkaline side. Correction of the pH had some effect in restoring capacity for germination and growth in the early stages, but the effect disappeared as the medium became more stale. Within wide limits, the pH value of a staled solution is not the limiting factor in growth.

The pH limits and optima for germination and growth of *Fusarium* sp. and *Botrytis cinerea* depend on the medium. Inhibition of germination is not caused by lack of nutrient material. The toxic effect is partly due to the presence of thermolabile substances. Precipitation by alcohol deactivates some of the inhibiting factors. Filtration through collodion membrane is partly effective in restoring its germinative capacity to staled Richard's solution indicating that some of the toxic substances present are colloidal in nature.

A survey of the evidence indicating that Phytophthora should be merged with Pythium.

II. II. FITZPATRICK.

Attention is called to the fact that there is at present no known basis for the satisfactory separation of *Pythium* and *Phytophthora*. The researches of recent years show that these genera do not differ essentially in method of swarmspore discharge, the character mentioned by Butler as providing "the only absolute mark of distinction" between them. As no other tangible taxonomic character which might serve for their separation has been brought to light the attempt to maintain the two generic concepts results only in confusion. The writer, therefore, proposes the merging of the genera under the name *Pythium* which is the older. It is also recommended that the Pythiaceae consisting of the resultant genus and its nearest relatives be regarded as a distinct family of the Peronosporales. The Peronosporaceae differ in their simultaneous development of sporangia, in the production of more definite sporangiophores, in the absence of saprophytic tendencies, in their more typically deciduous sporangia, etc.

The production of conidia at night in species of Sclerospora. WM. H. WESTON, JR.

In two conidial *Sclerosporas* (*S. philippinensis* and *S. spontanea*), destructive to maize in the Philippines, the writer, in investigations conducted by the Office of Cereal Investigations, B. P. I., U. S. Dept. of Agriculture, found that conidia were produced only at night when the host surface was covered with dew or other moisture. In the case of a third species (*S. sacchari* T. Miy.), recently introduced into the Philippines where it is destructive to sugar-cane, material furnished through the cooperation of Mr. H. A. Lee of the Philippine Bureau of Science, exhibits the same peculiarity. The accounts by Butler and Palm suggest strongly that this condition obtains also in *S. maydis* (Rac.) But. of India, and *S. javanica* Palm of Java. From these five oriental species the cosmopolitan *S. graminicola* (Sacc.) Schroet., on which the genus *Sclerospora* was founded, differs sufficiently to raise the question whether it, as well, produces its conidia only at night in dew. The writer finds that it does, and he assumes this fundamental peculiarity will be found to hold in other conidial representatives of the genus. Realization that the *Sclerosporas* produce conidia only during favorable nights opens the way to the more complete knowledge of methods of dissemination and infection on which control of these destructive and hitherto baffling downy mildews must be based.

Heterothallism in Ophiobolus cariceti. R. S. KIRBY.

Monosporous cultures of *O. cariceti* (B. & Br.) Sacc. are of two distinct sexual types which when mixed in culture result in the formation of typical perithecia. These perithecia develop in four months either in petri dishes containing two per cent crushed wheat in agar or in test tubes on stems of wheat or *Melilotus alba*. On certain media such as potato agar, wheat agar, cornmeal and oatmeal agars a definite dark zone is produced along the line of union between colonies of different sexual types. No such lines are produced where colonies of the same sexual type meet. Monosporous cultures have retained their sexual types during hundreds of tests throughout a period of 20 months. Polysporous cultures during the same period have been segregated into sexual types which react sexually with each other or with monosporous cultures of the opposite sex type. Each of the 175 isolations of monosporous or polysporous origin have exhibited a definite sex type.

Biologic specialization of Puccinia graminis avenae. E. C. STAKMAN, M. N. LEVINE, AND D. L. BAILEY.

Puccinia graminis avenae Erikss. and Henn. consists of several biologic forms differing in infection capability on certain varieties of *Avena* spp. Four and probably five biologic forms of *P. graminis avenae* can be identified by their action on Victory (C. I. No. 1145), White Russian (C. I. No. 1614), and Monarch Selection. Victory is completely susceptible, White Russian very resistant, and Monarch Selection practically immune from one of the forms. A second form infects Victory and White Russian in much the same manner as Form I, but produces heavy infection on Monarch Selection. A third form attacks Victory and Monarch Selection very heavily and White Russian moderately. All the three varieties are extremely susceptible to a fourth form. In some cases the heterogeneous (X) type infection described for wheat rust developed on Monarch Selection, thus indicating the probable existence of a fifth form. The behavior of these forms has been consistent. Forms I, II, and V, so far, have been found quite widely distributed in North America. Form IV was collected in Sweden, while Form III came from South Africa.

(Cooperative experiments between the Office of Cereal Investigations, B. P. I., United States Department of Agriculture, and the Minnesota Agricultural Experiment Station.)

Biologic specialization of Puccinia graminis secalis. M. N. LEVINE AND E. C. STAKMAN.

As a result of inoculation experiments made by the Office of Cereal Investigations, United States Department of Agriculture, in cooperation with the Minnesota Agricultural Experiment Station, it has been found that *Puccinia graminis secalis* Erikss. and Henn. consists of several biologic forms, as is the case with *P. graminis tritici* Erikss. and Henn., *P. graminis avenae* Erikss. and Henn., *P. coronata* Oda., and *P. triticea* Erikss. As rye is cross pollinated, the study of the biologic specialization of *P. graminis secalis* is more difficult than is that with the other rusts for which pure lines of differential hosts may be obtained with comparative ease. However, even commercial varieties of rye, such as Rosen, Swedish, and Prolific, react sufficiently clearly to indicate that there are at least two and probably three distinct biologic forms of *P. graminis secalis*. One form is quite virulent, possessing the capability of attacking heavily all the three varieties; another form attacks Rosen very heavily. Prolific only moderately and Swedish very weakly; while still another form produces normal infection on Swedish and Prolific, but only weak infection on Rosen.

Strains of the leaf-rust of wheat, Puccinia triticina, in the United States. E. B. MAINS AND H. S. JACKSON.

Cultures of leaf-rust of wheat from various parts of the United States, sown on 200 varieties of wheat, have shown that this rust is not uniform but consists of at least 12 strains differentiated by their action on 7 varieties, Malakof (C. I. No. 1898), Mediterranean (C. I. No. 5776), Democrat (C. I. No. 3384), and 4 unnamed spring wheats, C. I. Nos. 3756, 3778, 3779, and 3780. As a number of the cultures showed themselves to be mixtures, it was found advisable to make the final study from cultures started from a single spore, and 18 of these have been studied. As a result, 24 wheat varieties in addition to the 7 listed above have been found to be differential hosts, in the seedling stage under greenhouse conditions, to one or more strains of the leaf-rust. These are divided as follows: *Triticum aestivum*, winter 7, spring 5; *Triticum durum* 5; *Triticum dicoccum* 3; *Triticum spelta* 2; *Triticum monococcum* 1; *Triticum polonicum* 1. No variety was found absolutely immune from all strains and none of the cultures so far tested has been found to produce a high degree of infection on all these varieties. There seems to be more or less mixture of rust strains in the field but oftentimes different ones predominate in different sections. Some wheats, especially of the Turkey type, have shown high resistance in the field while showing considerable susceptibility in the greenhouse. (Cooperative investigation between Purdue Agriculture Experiment Station and Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.)

Wheat smut investigations in Kansas: Report of progress 1920-21. C. O. JOHNSTON.

Kanred, one of the principal varieties of hard winter wheat grown in Kansas, has been shown to be resistant to *Tilletia tritici* (Bjerk.) Wint. One year's results indicate that it shows resistance to this species of smut under Kansas conditions. In contrast to this, however, two years' results show that Kanred exhibits no more resistance to *T. levis* Kuehn, the species commonly found in Kansas, than Turkey, Kharkof, and other hard winter wheats. Soft red winter wheats, as a group, seem to be somewhat more susceptible to *T. levis* than do the hard red winter varieties.

Later dates of planting show consistently higher percentages of bunt than the earlier plantings, the amount of smut gradually increasing in consecutive plantings from September 15 to November 1, after which the percentage of infection rapidly declines.

Experimental data show that under conditions in eastern Kansas, spores of *Tilletia levis*, falling on the soil during August, do not seem to be a factor in producing bunt infection. This is perhaps not true for western Kansas where summer fallowing is practiced and summer rainfall is less. Further investigations are being conducted to prove this point.

Cytological studies on the infection of wheat seedlings by Tilletia tritici (Bjerk.) Wint. H. M. WOOLMAN.

Cytological studies conducted by the Office of Cereal Investigations, United States Department of Agriculture, in cooperation with the Oregon Agricultural Experiment Station, show that *Tilletia tritici* enters the epidermis of the coleoptile of both susceptible and resistant wheat plants grown under conditions for maximum infection, but that in highly resistant varieties it develops no further. Inhibiting factors evidently are active in or just beneath the epidermis. In varieties slightly less resistant no distinction from highly susceptible varieties can be observed until the plants are about ten days

old, this period being the time required by the fungus to pass into the permanent tissues of the plant, after which the suppressive factors evidently are in action. Cytologic examination of plants of this second or slightly less resistant class when 60 days old reveals only the same percentage of infection as is visibly developed in mature plants of the same lot.

In the epidermal cells the fungus hyphae are gram-negative, but the adjacent cell walls and cytoplasm are gram-positive. The hyphae are inclosed in a sheath of undetermined nature. In the interior of the coleoptile and in the true leaf sheaths the hyphae are both inter- and intracellular, gram-positive, and produce no observable change in the adjacent tissues. The same is true in the axis of the plant except that it is strictly intercellular.

Control of stinking smut of wheat (Tilletia levis) with dust treatments. G. H. COONS.

Copper carbonate, copper sulphate and lime dusts, using heavily smutted winter wheat, have given the following results in comparison with the standard formaldehyde treatments in stinking smut control:

Treatment	Sound heads per row	Smutted heads per row	Per cent of smut	Stand per cent
Control	568	495	46.	100
Dry formaldehyde	925	29	3.	98
Wet formaldehyde	743	0	0	97
Soak and skim formaldehyde method	900	5	0.5	97
Copper sulphate and lime dust, 2 oz. per bu.	1080	42	3.7	97
Untreated	500	408	44.	100
Copper carbonate, 4 oz. per bu.	850	23	2.6	98
Untreated	640	120	15.7	100
Copper carbonate, 2 oz. per bu.	823	12	1.4	97
" " 4 " " "	801	20	2.4	95
" " 8 " " "	844	8	0.9	90
" " 16 " " "	651	0	0	90
Untreated	320	160	33.3	100
Copper sulphate and lime dust, 2 oz. per bu.	555	27	4.6	98
" " " " " 4 " " "	570	24	4.	97
" " " " " 8 " " "	630	17	2.6	93
" " " " " 16 " " "	521	23	4.2	90
Control	255	203	44.	100
Copper sulphate dehydrated, 2 oz. per bu.	526	17	3.1	85
" " " 4 " " "	460	21	4.3	80
" " " 8 " " "	550	3	0.5	80
" " " 16 " " "	500	11	2.1	80
Sulphur, superfine, 2 oz. per bu.	457	76	14.	100

Considering the heavy smut production in the controls and the marked reduction brought about by both copper carbonate and copper sulphate and lime dusts, it is believed that use of these with fairly clean grain would give satisfactory stinking smut control. Tests with oats were not satisfactory in smut control.

Modified and simplified hot-water and vapor treatments for the control of loose smut in wheat, with special reference to seed injury. V. F. TAPPA.

Experiments conducted during the past two years by the Office of Cereal Investigations, United States Department of Agriculture, involving 11 seed lots of wheat, show on an average a reduction in yields of approximately 12 per cent when the seed was treated by the modified hot-water method, which commonly is recommended for controlling loose smut. Conclusive data show that this reduction in yields is due to a high percentage of seed killing caused by the treatment when the seed coats are broken over the embryo. The modified treatment simply retards germination when the seed coats are uninjured. Practically all of the breakage in seed coats results from machine thrashing. This has been shown by soil germination experiments in which treated seed carefully hand thrashed showed little or no injury while machine-thrashed seed was severely injured. The average germination of treated seed from 25 lots of the 1922 crop was about 40 per cent less for machine-thrashed than for hand-thrashed seed.

A simplified hot-water treatment has been devised which effectively controls loose smut of wheat reducing yields. A vapor treatment also has been employed successfully.

Organic mercury seed disinfectants. W. H. TILDALD AND J. W. TAYLOR.

Organic mercury compounds have been recommended in Germany since 1914 for the control of seed-borne diseases. Since 1920 a number of these compounds, including liquid and dust treatments, produced by American chemists, and Uspulun from Germany, have been tested by the Office of Cereal Investigations, Bureau of Plant Industry, Department of Agriculture, along with formaldehyde, hot water, copper sulphate-lime, and copper carbonate, to determine their effect on germination, vigor, and smut control of the small grains. The dusts, including copper carbonate, were less effective than liquids in controlling barley and oat smuts. The most promising liquid treatments used are Chlorophol, Uspulun, Corona Compound No. 620, and Semesan. Chlorophol and Uspulun were equally efficient. Corona No. 620 in less extensive experiments was apparently better than these, while Semesan compared favorably with them. These materials caused improved germination in greenhouse and field, increased field stands, and controlled smuts excellently. Barley and oats treated with Chlorophol, the only mercury compound used on seed sown in varietal plots, yielded better than did plants from seed of the same lots treated with copper carbonate or any other treatment. Copper carbonate proved best for wheat bunt. The mercury treatments look promising for the control of barley and oat smuts.

*Germination of the spores of timothy smut (*Ustilago striiformis* (Westl.) Niessl).* W. H. DAVIS.

Published results indicate that conditions for the successful germination of the spores of this smut parasitizing timothy (*Phleum pratense* L.), June grass (*Poa pratensis* L.), redtop (*Agrostis palustris* Huds.) and orchard grass (*Dactylis glomerata* L.) have not been understood. However, the writer has found that these spores germinate when properly after-ripened. Observations of bi-weekly germination tests continued through a period of two years may be summarized:

1. Conditions for the successful germination and the behavior of the germinated smut spores from each host are similar.
2. The smut spores pass through an after-ripening period varying from 180 to 265 days.

3. Period of germinability, 53 to 210 days; average, 70 days.
4. Nourishing solutions and decoctions are of no visible value in forcing the germination of these smut spores.
5. After-ripened spores germinate either floating or submerged in distilled water but do not germinate when placed on damp surfaces.
6. Temperatures: minimum, 10° C.; maximum, 35° C.; optimum, 22° C.
7. The fully developed promycelium may be septate and bear one branch from each cell.
8. A few promycelia bore typical sporidia at each cross-wall.
9. Spore germinations show that the parasite on each of the above hosts is an *Ustilago* and not a *Tilletia* as sometimes reported.

Mosaic and leaf roll of the potato in the northwest. GEO. K. K. LANK.

Mosaic is practically everywhere present in the Northwest, occurring in the leading varieties, Idaho Rural (generally 20-40 per cent), and Russet Burbank (10-20 per cent). The only stocks relatively free (5 per cent or less) are those introduced early into sequestered localities. During 1922 aphids were first found in potato fields after three years search. They appeared abundantly during late August and September in those northern sections considered the best sources for seed.

Infectious leaf-roll is most plentiful in an area embracing northeastern Washington, northern Idaho and western Montana, affecting primarily the Russet Burbank (15-20 per cent). It has increased decidedly during the period 1919-1922 and is spreading.

Observations (1919-1922) indicate that low humidities, high temperatures, and intense sunlight which characterize the growing seasons of the semi-arid and arid west cause not only such pronounced masking of mosaic and other virus disease symptoms, but also such marked changes in healthy plants that the symptomatology determined in the humid sections and the methods of field inspection used there are inadequate in the west. This is especially true under "dry-land" conditions. Determination of the responses of both healthy and diseased potato plants to these environmental factors is imperative before field inspection and seed certification in the west can be put on a firm basis.

Potato mosaic masking at high altitudes. H. C. MACMILLAN.

In the Rocky Mountain and other western potato growing sections the mosaic disease of potatoes has seldom appeared conspicuously in the generally characterized form. Some factor or set of factors masks the symptom of mottling. In potatoes grown at an altitude of 8000 to 8500 feet mottling is entirely absent. At lower altitudes the symptom of mottling is found intermittently. Potatoes newly infected with mosaic and grown under a louvered shelter constructed of wide strips of white cloth, and entirely open on the north side, developed conspicuous mottling, as compared with plants from the same seed tubers grown in the full sunshine and on which no mottling developed. Conditions in the shelter including temperature were practically identical with those outside, except that no sunlight fell on the plants. It is believed that the large amount of short wave-length light in the sunlight at high altitudes stimulates chlorophyll production in mosaic diseased cells, the effect being to mask the common symptoms. Some antiseptic action is possible. The life of the mosaic plants is longer under these circumstances. Non-mottled mosaic plants transferred from high to low altitudes readily develop mottling.

Spindling-tuber and other degeneration diseases of Irish potatoes. H. S. SCHULTZ AND DONALD FOLSON.

Transmission of spindling-tuber disease, commonly designated as "running-long" or as a type of "running-out," has been obtained with tuber grafts, vine grafts, leaf-mutilation inoculation, and aphids. Symptoms are erect and spindling stalks, smaller, more erect and somewhat darker green leaves than in healthy foliage, and spindling, spindle-shaped tubers (instead of flat, oblong tubers as in healthy (Green Mountains) with more numerous and more conspicuous eyes and with a smoother skin and consistency less firm than in healthy tubers. Spindling-tuber is in the leading commercial varieties in all percentages of incidence; spreads about as readily as mosaic; infects hills in part, resulting in diseased and healthy progeny from the same hill or even from the same tuber, as in mosaic and leafroll; and reduces the yield considerably. In addition to carrying mild mosaic, leafroll, and spindling-tuber, aphids transmit leaf-rolling mosaic, severe mosaic, and unmottled curly-dwarf. Leafrolling mosaic forms a curly-dwarf when in combination with spindling-tuber. Severe mosaic is more injurious and infectious, and causes current-season symptoms oftener and sooner than mild mosaic or leafrolling mosaic. If isolated from other degeneration diseases, mild mosaic strains will not acquire different symptoms. Mosaic and leafroll, together, or other degeneration disease combinations, may reduce tuber production affecting self-elimination. Transmission to healthy plants decreases as the distance from diseased plants increases, and fluctuates seasonally and regionally with aphid infestation.

Spindling tuber or marginal leaf-roll. KARL H. FERNOW.

The marginal leaf-roll disease of potatoes was recognized as a disease in 1921, when tuber units showing a large percentage of marginal leaf-roll were seen. The most striking characteristic of the disease is the elongation of the tubers which is often extreme. The vine shows an upright habit and is frequently dwarfed. The leaflets are slightly rolled at the margins, which are often crinkled, and are somewhat smaller than normal but do not show the extreme rolling which occurs in true leaf-roll. The stolons are not shortened as in true leaf-roll and there is no phloem necrosis. The examinations for phloem necrosis were made by Dr. Artschwager. The yield is considerably reduced. That the disease is transmissible is shown by the fact that among tuber units having a common ancestry some had degenerated while others were normal. A healthy plant on which a diseased scion was grafted produced a typically elongated tuber. The progeny has not been grown. The disease has been observed in Rurals, Green Mountains and Cobblers. The disease was previously mentioned by Quanjer. He noted the absence of phloem necrosis but not the elongation of the tubers.

A new host for potato mosaic. KARL H. FERNOW.

Twelve plants of the Apple of Peru, *Nicandra physaloides*, which were rubbed with crushed leaves of mosaic potato plants all developed distinct mottling. Four plants rubbed with leaves of mosaic tomato, four with leaves of mosaic tobacco, two with leaves from a mottled burdock plant and two with their own healthy leaves remained healthy. The leaves of two *Nicandra* plants which had become mosaic following inoculation from potato were used to inoculate two more *Nicandra* plants which later showed mosaic. Under favorable conditions the time elapsing between inoculation and the appearance of mosaic is two weeks or less. One uninoculated plant showed mosaic at the time the first inoculations were being made. A plant which was rubbed with leaves

from a mosaic *Physalis pubescens* plant also developed mosaic. Attempts to transmit the disease back to potato have so far given negative results. The progeny of inoculated potato plants has not yet been grown.

The occurrence of protozoa in plants affected with mosaic and related diseases. RAY NELSON.

Using modern cytological methods, protozoan killing and fixing solutions and protozoan stains, an intensive study has been made of bean mosaic, clover mosaic, tomato mosaic and potato leaf-roll. Definite protozoan organisms have been demonstrated constantly associated with these diseases and are located mainly in the sieve tissues. The bean and clover organism is a biflagellate, elongated protozoan whose generic position is near *Leptomonas*. It apparently is a flagellate of new generic rank, since the location of the flagella is unlike that belonging to any other described genus. The organisms found in mosaic tomato plants are apparently trypanosomes, or are closely related to this genus. They have been found only in the sieve tubes. Some individuals are 30μ or more in length, while very small forms are present which are less than 0.5μ wide and 6μ long. In the sieve tubes of potato leaf-roll plants, organisms have been found which more closely resemble trypanosomes than any other form. They are very variable in size, the smallest individual measured being less than 1μ wide and 12μ long. They are closely associated with the nucleus, in many cases actually coiled around it. All of the above organisms lie in a plane parallel to the longer axis of the host cell and have been demonstrated only in longitudinal sections.

Intracellular bodies associated with the rosette disease of wheat. H. H. MCKINNEY, SOPHIA H. ECKERSON, AND R. W. WEBB.

Microscopic studies on the rosette disease (formerly called take-all, so-called take-all, and foot-rot) of wheat revealed unusual intracellular bodies in the basal tiller tissues of plants showing early stages of the disease. These bodies also were found in wheat leaves showing a mosaic-like mottling which occurs not entirely on rosetted plants and on adjacent plants free from rosette and on plants of certain varieties which are apparently immune from the rosette disease. They have not been found in plants free from rosette and the mosaic-like mottling.

These bodies may be closely applied to the host nucleus, sometimes completely surrounding it, or they may be separated from it. They range in size from smaller to considerably larger than healthy wheat nuclei. In shape they vary from spherical to irregular and elongated forms. While these bodies resemble in certain particulars those described by Kunkel in connection with corn mosaic, they resemble more closely those described by Iwanowsky in connection with tobacco mosaic and by Lyon in connection with the Fiji disease of sugar cane.

The symptoms of the rosette disease of wheat bear certain resemblances to the symptoms of corn mosaic as described by Kunkel. (Cooperative investigations by Wis. Agr. Exp. Sta. and Cereal Investigations, B. P. I., U. S. Dept. Agr., with informal cooperation from Dr. C. A. Kofoed, University of California and from the Thompson Institute for Plant Research.)

*Intracellular bodies associated with a "mosaic" of *Hippeastrum johnsonii*.* H. H. MCKINNEY, SOPHIA H. ECKERSON, AND R. W. WEBB.

Studies made of free-hand sections and embedded material from the leaves of *Hip-*

peastrum johnsonii Bury, showing a mosaic-like mottling, revealed interesting intracellular bodies which were usually associated with the host nuclei.

In certain respects these bodies resemble the intracellular bodies found by the authors in connection with the rosette disease of wheat, but in general they are more nearly like those described by Kunkel in connection with corn mosaic, and apparently like those described by him in connection with the mosaic of *Hippeastrum equestre*.

As yet these bodies have not been found in the normal green areas of the leaves. In mottled tissue the bodies are found in the epidermal cells or in cells just below the epidermal layer. In the material thus far examined the bodies are present in about 5 per cent of the cells.

The nature of these bodies and likewise their exact relation to the mosaic-like leaf mottling is not known.

(Cooperative investigations by Wis. Agr. Exp. Sta. and Cereal Investigations, B. P. I., U. S. Dept. Agr.)

Temperature studies in mosaic diseases. B. T. DICKSON.

The roots of well-grown healthy and mosaic diseased tobacco plants were maintained in a constant temperature chamber at 37.5° C. (99.5° F.) while the stems and leaves were kept in an atmosphere of 21° C. (70° F.). They were given water at 37.5° C. daily. An ascending temperature gradient from soil to stem growing-point was thus developed. In 5 days the developed leaves became much darker green, especially noticeable in the hypoplastic areas of diseased leaves. In 10 days hypoplastic areas were dead in the lowermost leaves, but newly-developed leaves still showed marked color differentiation. Healthy and newly-inoculated seedlings were also tested. The first new leaves showed color differentiation, but were darker than at ordinary sap temperature. Later leaves were poorly developed but there was no cessation of infectivity.

Temperature studies in mosaic diseases. B. T. DICKSON AND E. G. HOOD.

The authors are making a series of tests to determine whether there is subnormal or supernormal temperature in mosaic-diseased tobacco, potato, bean, clover, etc. plants. Thermocouples of copper-constant wire with Type K Potentiometer, enclosed lamp and scale Galvanometer and Weston Standard Cell were used. The plants were tested in a constant temperature chamber. No constant temperature difference has been obtained as yet. More delicate couples are under construction and the tests will be continued.

Mosaic disease of sweet potato.—GEORGE F. WEBER.

Recent observations and experimental data concerning sweet potatoes show a diseased condition similar in many ways to the mosaic of other plants. Diseased sweet potato plants were first observed in a ten acre field where actual counts showed six per cent of the plants affected. The yield is reduced to less than ten per cent of the normal. The characteristic symptoms are: Internodes decidedly shortened; thus the nodes bearing leaves are close together, affected plant showing a decided rosette appearance. The petioles are somewhat shorter and thicker than those of normal plants. The petiole at point of union with the blade is often flattened. The larger veins branch out into the blade abnormally suggesting fasciation and appear much wider and more prominent than the veins in normal leaves. The tissue between the veins is of a uniform green color, decidedly raised or sunken, forming pouch-like areas. There is no mottling, in the sense that leaves of cucumber and tobacco plants are mottled by

mosaic. The blades are crinkled along the edges, curled backward and in severe cases malformed. Twenty healthy plants were inoculated in various ways with the juices extracted from twelve different diseased plants but symptoms of the disease did not develop.

The location of the crown-gall organism in its host tissues. A. J. RIKER.

In inoculation studies of crown-gall on tomato stems it was observed that needle wounds immediately released liquid which flooded the neighboring intercellular spaces and formed water-soaked areas about the punctures. As the galls developed, they were found to coincide closely in outline with these water-soaked regions. Galls of different sizes were induced experimentally by varying the sizes of the inoculating needles and consequently those of the water-soaked areas. Expressed tomato sap was found to exert a positive chemotactic influence on the bacteria. They migrated through water-soaked tissue and produced galls several centimeters from the point of inoculation. The bacteria were observed consistently *in situ* in an intercellular position both in paraffin and free-hand sections from five series of inoculations which were examined at two day intervals until distinct hyperplasia, hypertrophy, and vascular elements developed. They appeared to be present in larger numbers than has previously been supposed and to exert their stimulating influence from a position between the cells.

Some morphological responses of the host tissues to the crown-gall organism. A. J. RIKER.

Studies of crown-gall made on tomato have shown that the causal organism begins its relations with the host in the liquid released by the wound. This liquid was found to flood the neighboring intercellular spaces. From this position the bacteria stimulated some of the nearer host cells to divide into smaller cells forming a region of hyperplasia. Beyond this region the cells often reacted by swelling to form a region of hypertrophy. When the bacteria were distributed through the intercellular spaces for some distance in a more or less straight course, the subsequent division of the surrounding cells formed a "tumor strand." When inoculations were made near the growing tips of sweet pea and sunflower plants which have condensed buds, occasionally "secondary galls" developed at some distance from the points of inoculation while "tumor strands" developed in the intervening region. In such plants as those mentioned the flooding of the intercellular spaces for a few millimeters in the region of elongation would enable the bacteria to separate themselves from the point of entry by a number of internodes. When the process of elongation took place, the secondary galls would be separated from the point of inoculation by distances varying with the length of the internodes. Heavy infection near the growing tip may, however, prevent the normal elongation of the internodes.

Time of apple blotch infection for 1922 in southern Pennsylvania. R. C. WALTON AND C. R. ORTON.

Bagging experiments on unsprayed Smith Cider trees in Adams County, Pennsylvania, prove that infection by *Phyllosticta solitaria* started very early in the spring of 1922. Young apples bagged continuously from May 23 (17 days after the petals fell) until August showed 64 per cent blotch, indicating that initial infection occurred prior to May 23. Infection on petioles and leaves was first noticed on the bagging date (May 23) but was not noted on the unbagged fruit until June 13 when the largest spots measured 4 mm. in diameter. Because of the slow development of the disease, these spots must have been visible for at least a week prior to this date.

According to Roberts, "*Phyllosticta solitaria* grows slowly and is not perceptible on the fruit until 3-6 weeks after infection has taken place." If this holds true for Pennsylvania conditions and granting that fruit infection was visible by June 6, then according to Roberts the latest date when infection occurred was about May 16 which is only 10 days after the date of petal fall. This brings the time of probable initial infection on fruit at least 10 days prior to the time usually recommended for applying the first spray.

New aspects of apple blotch control. MAX W. GARDNER AND H. S. JACKSON.

In Indiana, apple blotch in the canker form is introduced into nurseries on the seedlings and into young orchards on the nursery stock. Cankers occur abundantly on budded seedlings both above and below the bud and on susceptible stocks such as Northwestern. Scions from susceptible varieties may carry the disease. Trunk and limb cankers are of common occurrence in young orchards of such varieties as Oldenburg and it has been found that these cankers can be easily cut out with a sharp knife in early spring without injury to the underlying cambium. Healing occurs rapidly and no wound dressing is necessary. There is little danger of reinfection if all of the discolored bark is removed and if the cut is made at least a centimeter in advance of the visible margin of the canker, particularly at each side. Thorough-going canker eradication combined with the blotch sprays to prevent new cankers is advisable in young orchards. The application of blotch sprays in nurseries and the use of blotch-free stocks and scions is much to be desired.

Apple scab control in Massachusetts. WEBSTER S. KROUT.

Under conditions most favorable for scab infection, almost perfect control of apple scab on the McIntosh variety was obtained by the writer. Investigations have extended over the last two years and have been along two lines: (a) a study of the causal fungus, *Venturia inaequalis*, and (b) a study of control measures. Each year the time of ascospore discharge has been determined in order that the pink application might be timed correctly. Temperature, relative humidity and rainfall records have been accurately recorded for correlation with the occurrence and prevalence of scab.

Field work was carried on in six different orchards, and data were taken from over 100,000 apples. Best results were obtained with a 3-10-50 home-made Bordeaux mixture for the pink application, followed by liquid or dry lime-sulphur for succeeding sprays. The results indicate that dry lime-sulphur is as effective as the liquid form against scab.

In 1922 dusts were practically as effective as the spray materials. Sulphur dusts and copper-lime dusts were tested. The former was more satisfactory than the latter as it did not russet the fruit or burn the foliage.

*The importance of the Phoma stage of *Mycosphaerella rubina* causing spur blight of raspberries.* A. G. NEWHALL.

The imperfect stage of *Mycosphaerella rubina* (PK.) Jacq. has been proved to be a normal part of the life cycle of this pathogene. In the latitude of southern Minnesota and New York the perithecia mature in the spring and discharge their spores by the middle of June. Mature pycnidia have been found in primary cane lesions the last week of June and in leaf spots the first week of July. This Phoma stage plays an important role by initiating secondary cycles throughout the remainder of the growing

season. The connection between the perithecial and pyrenidial stages of *Mycosphaerella rubina* has repeatedly been demonstrated in single spore cultures on Melilotus stems. Furthermore, suspensions of ascospores inoculated into young raspberry canes likewise gave characteristic lesions in which the Phoma stage developed.

Aerial galls of peach. J. A. McCLINTOCK.

In the fall of 1920 peach seeds were planted on a piece of land 125 yards wide and 350 yards long which had been cleared of oak timber in 1916. This land is joined on three sides by standing timber mostly oak, some of which have large galls on their trunks.

The peach seedlings were budded June 25-30, 1921, and 10 days later they were topped at the bud. About 4000 trees out of a total of 80,000 developed galls at the wound made by topping. The roots were free from galls. In the fall of 1921 peach seed were again planted on this land and in 1922 budded and topped about the same time as in 1921. A careful search November 10, 1922 disclosed three trees bearing aerial galls and only two of these were at the wound made by topping. The growing season of 1921 was wet throughout while in 1922, it was dry. The grower thought the peach galls must have come from the galled oaks. Cultural studies of these peach galls are being made.

Melanose and stem-end rot of citrus fruits. O. F. BURGER.

Melanose is caused by the fungus *Phomopsis citri* Faw. This fungus lives in the dead wood, and in the spring of the year the spores are washed down on the fruit and leaves by the rain, where they germinate and cause the common form of russetting known as melanose. Leaves four weeks old are immune to the attack of the fungus. In preliminary work I found that the grapefruit become immune about the middle of May. When the fruit begins to ripen they are again attacked by the fungus, which at this time causes a decay of the fruit known as stem-end rot. This decay always occurs after the abscission layer is formed. The abscission layer forms a natural opening for the fungus to enter. It has been determined that the fungus cannot penetrate the rind. By inoculating green or ripe fruit with *Phomopsis citri* Faw. decay takes place. In March and April, 1922, spraying experiments were begun to control melanose. Trees were sprayed with a 3-3-50 Bordeaux solution, plus 1 per cent of oil emulsion, ten to twenty days after the blossoms dropped. All the data have not been collected; but what evidence we have indicate that melanose is controlled by this spray.

Foreign studies of white pine blister rust. PERLEY SPAULDING.

Eight months have been spent in the various European countries studying the white pine blister rust. Except in Switzerland and Germany, *Pinus strobus* occurs in relatively small numbers and is really an ornamental rather than a timber tree. Moir in 1920 added *Pinus koraiensis* to the known white pine hosts of *Cronartium ribicola*, leaving but four species not known to be attacked. Since then *P. strobiformis* has been inoculated in the greenhouse and *P. balfouriana* has been doubtfully reported as infected in Europe. During the present studies *P. albicaulis* was found in England bearing pycnia and one aecium. The oldest *P. strobus* seen in an infected area were in Switzerland and ranged upwards to 118 years in age. Trees of this species were seen in various countries, ranging in age from 4 to 118 years, that were killed or being killed by the blister rust. There is not the slightest doubt that the largest and oldest trees can and will be killed by it. The older trees are killed by girdling of the trunk in the lower part of the crown. Exuded pitch is much more conspicuous on the cankers in Europe than in America.

White pine blister rust in the Northwest. HAVEN MITCALF.

The oldest infected (1910) pine wood found at Vancouver, B.C., indicates that Vancouver was the place first infected. Records are clear as to importation of *Pinus strobus* from Europe to Vancouver at this time. On the other hand it is not impossible that there were independent introductions of the disease on *Ribes* as well as pine at other points, both in British Columbia and in Washington. Both hosts were introduced independently at many places before quarantines were in effect in either the United States or Canada, and it would be strange if some of these introductions were not diseased. The disease now extends north (on pines and *Ribes*) to the limits of *Pinus monticola*, east (on pines and *Ribes*) to Beaton, B. C., and south (on *Ribes*) to within 25 miles of the Columbia River at the coast, thus occurring within a triangle of country 425, 320 and 315 miles on a side. The heaviest spread has been northward, due to climatic conditions and greater abundance of cultivated black currant in British Columbia. Serious local damage to *Pinus monticola* has already occurred. This host appears to be somewhat more susceptible than *P. strobus*, but aside from this fact the disease apparently behaves much as in the East.

White-pine blister rust infection through grafted roots. MINNIE W. TAYLOR.

Recent field observations have raised the question as to whether the white-pine blister rust (*Cronartium ribicola* Fischer) might spread from diseased to healthy trees through the roots. Abundant well-developed aecia of *Cronartium ribicola* were found on a root of *Pinus strobus* growing under several inches of leaves and loam. Twelve other roots were examined to determine how far infection might extend down or along the roots. The roots ranged from 5 inches to 28 inches in length and hyphae and haustoria occurred in eight of them, extending from $4\frac{3}{4}$ inches to 16 inches down the root. Pycnial layers were observed in four of the roots, the lowest depth being 10 inches. Grafted roots occurred in two out of three specimens collected to show this feature especially, and in two out of five specimens selected for determining the extent of infection down the root. The grafted roots of four pairs of trees were studied; in each case one of the paired trees was infected. Hyphae and haustoria were found in the graft tissue in three cases. In two of the specimens the infection extended through the graft tissue well up the trunk and down into the root of the second tree, thus supporting the original theory that infection might spread by means of grafted roots.

Studies on the aecial stages of Cronartium ribicola and Cronartium occidentale. REGINALD H. COLLEY AND MINNIE W. TAYLOR.

Morphological differences in spore markings and margins are slight but consistent; the peridial cell arrangement and markings are characteristic; and the "concomitant cell," an element midway between the spore and the peridial cell in *occidentale*, appears to have no homologue in *ribicola*. Concomitant cells measure (500) $17.90 \pm .072 \times 21.96 \pm .078$, standard range $15.52 - 20.28 \times 19.36 - 24.56$. The two species can be readily separated on a basis of morphological and dimensional differences.

Statistical analysis of *Cronartium ribicola* and *Cronartium occidentale*

Aeciospores (2000)*	M. $18.1 \pm .03 \times 24.1 \pm .04$	$19.1 \pm .04 \times 26.8 \pm .05$
	R. $16.0 - 20.2 \times 21.6 - 26.7$	$16.7 - 21.5 \times 23.7 - 29.9$
Aeciospore markings, surface view (500)	M. $1.1 \pm .07 \times 1.5 \pm .09$	$1.3 \pm .08 \times 2.3 \pm .2$
	R. $.9 - 1.3 \times 1.2 - 1.8$	$1.1 - 1.6 \times 1.6 - 2.9$

Peridial cells, outer layer,	M.	$29.2 \pm .1 \times 42.2 \pm .2$	$19.1 \pm .1 \times 27.4 \pm .1$
section view (500)	R.	$21.9 - 33.5 \times 35.0 - 49.3$	$15.6 - 22.5 \times 23.2 - 31.7$
Peridial cell walls,	M.	6.8	4.7
outer wall (500)	R.	5.3 - 8.2	3.7 - 5.6

* - Unites measured, M. = mean, R. = standard range based on coefficient of variability.

A noteworthy case of resistance to the chestnut bark disease. J. FRANKLIN COLLINS.

Records and photographs of a sprout of an American chestnut tree in Pennsylvania which in 1912 was 1.2 inches in diameter and showed several places badly diseased by the chestnut bark disease fungus. In ten years this sprout had increased to approximately 7 inches in diameter, and certain lesions caused by the disease, which were very evident in 1912, showed little external evidence of disease in 1917 and later. The sprout always was and still is badly diseased in various places. The fact that a sprout of American chestnut but little more than an inch in diameter, and apparently girdled at the base in 1912, should have survived for ten years and increased to 7 inches in diameter seems noteworthy. Lantern slides will show views of the base of the sprout taken in 1912, 1917, 1920 and 1922.

The Melanconis disease of the butternut. ARTHUR H. GRAVES.

This is characterized in its first stages by the appearance of dead limbs besprinkled with small black acervuli of the causal fungus, *Melanconis juglandis* (E. & E.) comb. nov. Occasionally the acervuli develop spore horns, but are usually rounded or wartlike, and in wet weather like drops of thick ink. This conidial stage has been known as *Melanconium oblongum* Berk. The ascospore stage, which may appear later near these same pustules, has been known as *Diaporthe juglandis* E. & E., but by culture work this is now definitely proved to be the perfect stage of *Melanconium oblongum* Berk. Inoculation experiments, extending over a period of more than four years, have conclusively demonstrated that the fungus is a weak parasite. Entering usually through small twigs by way of a wound, the mycelium grows slowly down through the wood—faster if the tree is already weakened—to the main branch and finally to the trunk. Ordinarily the progress of the disease is so slow that the leaves fall one by one, not producing any striking wilting or blighting effects. In final stages affected trees have a marked stag-headed aspect. Diseased branches should be pruned off promptly some distance below apparent infection and the wounds tarred over or painted. After the fungus has penetrated the trunk no remedy is practicable.

The relation of an undescribed species of Pestalozzia to a disease of Cinnamomum camphora Nees & Eberm. NATHANIEL O. HOWARD.

Investigations have been made recently to determine the relation of fungi to a disease of *Cinnamomum camphora* Nees & Eberm., a malady which seriously threatens the production of raw camphor in this country. The disease is caused primarily by a thrips, *Cryptothrips floridensis* Watson. Certain camphor growers, however, regard the thrips injury as merely incidental to the attacks of a fungus, the latter being the really destructive organism. An undescribed species of *Pestalozzia* was found to be quite constantly associated with the thrips injury. Moreover, evidence was obtained indicating that the thrips is partly responsible for dissemination of the *Pestalozzia* spores. Inoculation experiments, however, conducted in the greenhouse upon camphor

plants entirely free from thrips indicate that, under these conditions at least, this *Pestalozzia* sp. is unable to attack the healthy tissue, but develops readily in dead portions of the host. It appears, then, that this fungus is a saprophyte or, at the most, a weak, wound-parasite upon *Cinnamomum camphora* Nees & Werm. and that control of the disease lies in the elimination of *Cryptothrips floridensis* Watson.

Progress in barberry eradication. F. H. KIMPTON.

In the barberry eradication campaign conducted cooperatively for five years by U. S. Department of Agriculture and 13 north-central States, marked progress has been made. During 1922, the survey covered 200 counties. This includes 16 counties in Minnesota and one in Iowa surveyed on State funds. In these counties, 186,672 bushes were located on 5,278 properties, and 696,517 bushes were removed from 5,799 properties. Of 275 counties previously surveyed, 201 were completely resurveyed in 1922, and 84,142 sprouts were found and removed. Experiments on chemical methods of eradication, begun in September, 1921, have yielded highly important results. Bushes properly treated with salt or sodium arsenite die without sprouting. Investigations on the distribution of rust spores by air currents are being conducted cooperatively with the War Department Air Service which furnishes airplanes and pilots.

During the five years, 1918-1922, practically all cities and villages of the 13 States have been covered once and resurveyed in part, and 475 counties have been completely covered. This includes 40 counties surveyed on funds furnished by States. In the 5 years, 2,066,541 bushes have been found in cities and villages and 3,740,102 on farms, making a total of 5,806,643 bushes found, of which 5,140,343 have been removed.

Eradicating the common barberry by means of chemicals. NOEL F. THOMPSON.

In connection with the cooperative barberry eradication campaign of the U. S. Department of Agriculture and 13 north-central States, it has been found that abundant sprouting from root fragments occurs under certain conditions when the bushes are dug. In the rolling, more or less wooded portions of these States, hundred of thousands of escaped bushes have developed from seeds carried by birds or animals. Under these conditions and especially in well-trained, more or less alkaline or limestone soils, root fragments sprout most abundantly. For these reasons the effectiveness of chemicals in killing the bushes is being studied. While the experiments have not been completed, certain promising results have been obtained.

More than forty different chemicals have been studied. Of these, two seem satisfactory from the standpoint of effectiveness, cost, and availability. These are salt (sodium chloride) applied at the rate of about 10 pounds per bush of average size, and a solution of sodium arsenite (1 lb. to 5 gal. water) applied at the rate of two gallons per bush. Salt may be applied at any period of the year. The sodium arsenite is effective when applied throughout the growing season, but its action when applied in the late fall and winter is not yet fully determined.

Pathological morphology of durum wheat grains affected with "black point." WANDA WENIGER.

For many years a disease of durum wheat, particularly of Pentad (D5), Monad (D1), Kubanka, and Arnautka, has occurred in North Dakota. The disease is characterized by "black pointed" grains, in which the embryo end is typically creosote colored. Some kernels are decidedly shrivelled as the result of early infection, but the majority are not noticeably underweight. Poor germination is common, and frequently a destructive.

The composition of corn seedlings at low and high soil temperatures is the reverse of that for wheat.

In the invasion of coleoptile and coleorhiza of seedlings, the parasite penetrates the walls of pectic materials apparently with little resistance and is both inter- and intracellular, whereas, it penetrates the cellulose walls slowly, digesting the middle lamella and thus remaining intercellular. All gradations between these two types of penetration occur in the intermediate soil temperatures.

(Cooperative investigations by Wis. Agr. Exp. Sta. and Cereal Investigations, B. P. I., U. S. Dept. of Agr.)

The production in culture of the ascigerous stage of Fusarium moniliforme. GRACE O. WINELAND.

For two years, the behavior of a number of single spore cultures of *Fusarium moniliforme* Sheldon isolated from corn has been under observation, particularly for the development of an ascigerous stage. Some of these cultures have produced peritheciium-like bodies under certain conditions, but no combination of environmental factors has been found that would induce the development of mature perithecia. Recently, mature perithecia of the Giberella type have been produced in abundance, but only by combining two strains of the fungus. In one experiment, strain Y 15, alone, produced only peritheciium-like bodies, but, when it was combined with Y 29 or S 1, mature perithecia developed along the line of contact of the two strains in a majority of the tubes and plates inoculated, while Y 29 and S 1, separately or in combination, produced neither peritheciium-like bodies nor perithecia.

Single germinating ascospores, from the Y 15 and Y 29 combination, produced microconidia in chains and 3-septate macroconidia typical of *Fusarium moniliforme*.

The strains mentioned differ somewhat morphologically, but all come within the limits of the new section Moniliform established by Sherbakoff.

(Cooperative investigations by the Wis. Agr. Exp. Sta. and Cereal Investigations, B. P. I., U. S. Dept. of Agr.)

Influence of temperature on the morphology of Fusarium spores. HILLEN JOHANN.

Experiments with the study of *Fusaria* indicate that the temperature at which the fungus is grown may affect the size, shape, and septation of the macroconidia. Cultures of a species representative of the section Roseum, whose optimum temperature for vegetative growth lies between 18° and 21° C., produce sporodochia in which 5-septate conidia predominate, averaging in size 62.2 by 3.4 μ when grown on various media in the laboratory at about 22° C. Under such conditions few 6- and 7-septate conidia are found. In similar transfers subjected to temperatures of 10° to 14° C., other conditions, except light, being the same, the spores are normal, but broader and less curved and a much higher percentage are 6- and 7-septate. The 6-septate conidia from these cultures average 67.25 by 4.32 μ . The effect of absence of light at low temperatures has not yet been determined. The presence or absence of diffuse light at room temperature appears to be comparatively unimportant.

Preliminary experiments with other sections of the genus *Fusarium* indicate that modification of the macroconidia may be obtained there also, although the results are less striking.

(Cooperative investigations by Wis. Agr. Exp. Sta., and Cereal Investigations, B. P. I., U. S. Dept. of Agr.)

The pathogenicity of Fusarium moniliforme Sheldon on cereals. A. W. HENRY.

Single spore cultures of *Fusarium moniliforme*, isolated from wheat seed from Missouri and from wheat sick soil at University Farm, St. Paul, Minnesota, proved virulently pathogenic to wheat (spring and winter), barley, field corn and sweet corn and also attacked rye and oats. Both cultures killed wheat seedlings growing on nutrient agar in large test tubes. They also killed or stunted wheat plants growing in sterilized soil inoculated with the organism in the greenhouse. The roots were rotted and the basal portion of the stems were sometimes attacked. The fungus was easily reisolated. On potato-dextrose agar, comparatively few macroconidia were produced but long chains of microconidia were abundant. Both spore forms were produced in abundance when moist sterilized wheat seed was inoculated either with cultures from single microconidia or from single macroconidia. The culture isolated from wheat seed and that from the soil differ slightly but both correspond closely with the description of *Fusarium moniliforme* Sheldon. Both cultures grew slightly between 5 and 7° C. and between 36 and 36.5° C. The optimum temperature for growth apparently lies near 30° C.

Corn root, stalk, and ear rot disease investigations in Kansas: Report of progress 1922.
L. E. MELCHERS AND C. O. JOHNSTON.

Varieties of white corn used in the investigations show the presence of an abundance of *Fusarium moniliforme*, while only occasionally a trace of *Diplodia zeae* and even less of *Gibberella saubinetii* occurs on the germinator. *Penicillium*, *Rhizopus*, and *Aspergillus* are much more common than *Diplodia* and *Gibberella*. *Fusarium moniliforme* is by far the most common and occurs to a greater or less extent on over 95 per cent of all the ears so far tested. This organism occurs externally on the grain in apparently 50 per cent of the cases. Up to the present no noticeable correlation can be seen between the occurrence of seedling injury on the germinator by *F. moniliforme* and the symptoms of root, stalk, and ear rot as it occurs in the fields of Kansas. These data have been taken chiefly from strains of corn that have been selfed for two or more seasons.

The occurrence of *Fusarium* and other "molds" on corn in Kansas as shown on the germinator is apparently not in itself sufficient or completely reliable to use as a basis for selecting or eliminating seed corn under Kansas conditions in view of our present knowledge.

(Cooperative investigations by Kansas Agr. Exp. Sta. and Cereal Investigations, B. P. I., U. S. Dept. of Agr.)

Relation of environment to infection of corn seedlings by Diplodia zeae (Schw.) Lev. HILLEN JOHANN, JAMES G. DICKSON AND GRACE WINELAND.

Five series of experiments conducted in the Wisconsin temperature tanks indicate that *Diplodia zeae* does not cause a destructive seedling blight of corn when healthy seed is inoculated with spores or mycelium and the seedlings are grown under favorable conditions. One series under early summer conditions and in medium wet soil showed no evidence of disease. In contrast, inoculated seedlings grown in large test tubes are killed in about two weeks. The fungus is capable of attacking the mesocotyl, especially under extremely high or low soil moisture conditions at temperatures ranging from 24° to 32° C. which includes the optimum temperature for the growth of the fungus. In wet soil at 28° C., 63 per cent of the plants showed severe browning of the mesocotyls. These plants were not much stunted but leaned considerably due to their weakened

mesocotyls. The effect of this type of injury on later development is not known as the plants were not carried far beyond the seedling stage. (Cooperative investigations by Wis. Agr. Exp. Sta. and Cereal Investigations, B. P. I., U. S. Dept. of Agr.)

Helminthosporium disease of rice. G. O. OCFEMIA.

In an earlier abstract (Phytopathology 12: 34. 1922), the disease caused by *Helminthosporium oryzae* Breda de Haan was called "Sesame spot disease of rice." The change of title is made in order to make the disease distinctly that of the rice and avoid possible confusion with a disease of *Sesamum indicum* L., caused by *Helminthosporium sesamum* Sacc.

Experiments were conducted on the relation of soil temperatures to germination of certain Philippine low land and up land rices and the production of disease from artificial inoculations. Rice germinated two to three days after sowing at 36° C. and 32° C., three to four days at 28° C., four to five days at 24° C., five to six days at 20° and 40° C., and eight to fourteen days at 16° C., with soil moisture 25 per cent of the water holding capacity of 27.5 per cent. Subsequent growth of seedlings is best at soil temperatures of 28° to 32° C. Infection takes place in soil temperatures from 16° to 36° C., but is most severe at 28° to 32° C. Certain Philippine and Louisiana low land rices sprout under 10 to 20 centimeters of water at temperatures of 20°, 24° and 28° C., in two weeks. Inocula applied externally on flooded rice did not produce infection. The causal fungus produced symptoms of the disease in artificial inoculation on 31 species of 23 genera of grasses.

Disease of flax caused by a species of Rhizoctonia. W. E. BRENTZEL.

A destructive and apparently undescribed disease of flax has been observed in a number of fields in eastern North Dakota. The disease occurred in somewhat definitely limited areas from a few feet across to more than an acre in extent. In these areas, practically every flax plant was infected. On individual plants, the infection first appears on the roots as small brown lesions just beneath the surface of the soil. Later, these lesions enlarge and extend upward to the cotyledonary leaves and also downward, covering an area measuring from 1 to 4 or 5 centimeters in length. In the later stages of the disease, the affected cortical portion of the stem is reduced to a dry pulp which sometimes breaks and splits in such a manner as to give a ragged appearance. The fungus enters the pith and spreads, but the extent of penetration has not been determined. The plants wither and die prematurely, taking on the general appearance of "wilt." The associated fungus apparently belongs to the genus *Rhizoctonia*. The fungus is pathogenic on flax but non-pathogenic on various other plants thus far inoculated. Its minimum, optimum, and maximum growth temperatures in culture are about 13, 26, and 35° C., respectively. (Cooperative investigations by the N. Dak. Agricultural Experiment Station and office of Cereal Investigations, B. P. I., U. S. Dept. of Agr.)

A disease of flax not previously reported in the United States. W. E. BRENTZEL.

During the past 4 years a disease of seed flax has been observed in North Dakota, which develops characteristic greenish-yellow to dark brown mottling on the stems, leaves, and capsules. During the past season the same disease was observed on fiber flax in Michigan by Mr. R. L. Davis, who sent specimens for identification. The disease is very similar to if not identical with that found in South America caused by *Phlyctaena linicola* Spog. The lesions on the stems vary in size and shape, depending

on the stage of development. At first they appear as dots, then lengthen and later widen until they extend around the stem. They may cover an area of one half to several centimeters in length, leaving irregular-shaped bands of green between lesions. The spores of the associated fungus are borne in pycnidia and are hyalin, practically cylindrical, straight or slightly curved, and measure about 1.5 to 3.0 by 21 to 31 μ . Artificial inoculations on different varieties of flax indicate that certain varieties are very susceptible, while others are somewhat resistant. (Cooperative investigations by the N. Dak. Agricultural Experiment Station and Office of Cereal Investigations, B. P. I., U. S. Dept. of Agr.)

Dutch potato varieties resistant to wart disease and internal brown spot. J. CORTWIJN BOTJES AND H. M. QUANJER.

The varieties tested for resistance to the wart disease were first grown during a series of years in isolated rows in order to free them through selection from leaf-roll, mosaic and other of the so called deterioration diseases. At the same time data have been collected on susceptibility to internal brown spot, on yields and other qualities.

The varieties thus prepared for the investigation have been planted in 1922 on ground infected with the wart disease between rows of a rather susceptible Dutch variety which served as indicator. The experimental plot was situated in the north of the country near the German frontier, which the disease has overlapped. The investigation extended over 33 Dutch, 7 German, 1 English and 2 Swedish varieties. A great difference in degree of attack was noticed. The varieties which remained free from wart infection were the following of the breeder Veerkamp; Favoriet and Triumph; of the breeder De Vries; Fonteyn, Kruisling and Lieuwe. Also one German variety of the breeder v. Kameke; Pepo remained free from the disease. As regards internal brown spot Favoriet is susceptible, Triumph is resistant, while the three other Dutch wart-resistant varieties are intermediate. These five varieties have all yellow flesh, which is preferred for consumption in Holland. The white fleshed German variety Pepo is considered only good for the starch factories.

Spraying and dusting of potatoes in 1922 at Chatham, Michigan. J. E. KOTILA AND G. H. COONS.

Large plots of Green Mountain potatoes with frequent controls and abundant replications have given results permitting evaluation of various types of foliage treatment. The results are as follows:

TABLE 1.

Potato spraying and dusting tests: Yields for treatment represent averages of 5 plots, the controls an average of 11 plots.

Treatment	Number of applications	Yield in bushels per acre		
		No. 1	Culls	Total
Control.....		160	40	200
Bordeaux mixture 4-4-50.....	6	241	43	284
Dehydrated copper sulphate and lime dust.....	6	192	41	233
Bordeaux mixture 4-4-50—nicotine 1-800.....	3	199	35	234
Dehydrated copper sulphate and lime plus nicotine 2½ per cent..	3	147	38	184

In this field hopperburn and early blight were the disease factors encountered. The differences in yield show the relative efficiencies of the various treatments. It is evident that Bordeaux mixture is far superior to Bordeaux-like dusts, and that its application brings high returns in increased yields.

Hot water and hot air treatments of potatoes. F. M. BLODGETT.

Hot water treatments of mosaic affected Bliss potatoes reported last year were continued with potatoes of the Rural group affected with leaf roll. The results on the killing of Rurals agree so closely with those for Bliss that they may be considered together. The equation given last year of the time-temperature curve for the killing of potatoes has the values of its constant terms slightly changed to fit as nearly as possible all these results and is: $\log_{10} t + .0955 X = 6.588$ in which t is time in minutes and X is temperature in degrees Centigrade. This equation is sufficiently accurate to warrant its use as a guide in experimental treatments of potatoes with hot water. All potatoes surviving the treatment still carried leaf roll. Surface injury and decreased sprouting appeared on potatoes treated about half the time required for complete killing. Black heart resulted at all temperatures between 40 and 100° C. in the time range just above that necessary for killing the potatoes. Preliminary experiments with dilute solutions of mercuric chloride produced curves which are parallel to the preceding and slightly below it.

Hot air treatments were tried with white sprout potatoes affected with mosaic. All potatoes surviving the treatment had mosaic. The relation of time and temperature found for killing potatoes by hot air may be expressed by the following equation: $(t - 8.425)(X - 34.5)^3 = 1057000$.

Studies on the causes of stem-end discolorations of potato tubers in North Dakota. WANDA WINIGER.

During the past three years cultural studies of stem-end discolored tubers from nearly every potato growing section of the state have been made with the view of determining the cause and nature of vascular discolorations. The tubers studied were mostly of the Early Ohio variety, all showing a distinct browning of the vascular tissues when cut across about one-fourth to three-eighths of an inch from the point of rhizome attachment. Cultures were made from tissue taken at an average depth of one-half inch from the point of attachment and at least three made from each tuber. Fusarium wilt was found associated with less than one-half of the discolorations, and was not, as is popular belief, the sole cause of discolorations. Many tubers, although showing a distinct browning, gave no growth of any parasitic organism, while others showing little browning gave the Fusarium wilt fungus. In a large percentage of tubers the blackleg bacillus was found, while in some both blackleg and wilt were present. These discolorations, whether yielding growths of Fusarium wilt, of blackleg, of both wilt and blackleg, or of no organism, were not distinguishable either in color or extent.

Ito's potato variety Ekishirazu in New York. DONALD REDDICK.

The potato variety Ekishirazu has been tested in greenhouse and field at Ithaca, New York. In a cool house it grows luxuriantly and produces large tubers. In a warm house it makes very poor growth and produces many small tubers. Both in 1921 and 1922 its growth in the field corresponded to that of the warm house. The variety has been subjected repeatedly, both under glass and out of doors, to extremely

favorable conditions for infection by *Phytophthora infestans*. Occasional small lesions have been produced but conidiophores on lesions of attached leaves have been found only once. The variety substantially bears out its name, "blight unknown," but it is susceptible to mosaic. Under cool conditions the variety produces great quantities of pollen. In conjunction with C. H. Myers, hybrids have been secured with Rural Green Mountain and Evergreen, the latter variety itself possessing marked blight resistance.

The relation of humidity to the infection of sweet potatoes by Rhizopus. J. I. JAURITZEN
AND L. L. HARTER

Only a small percentage of potatoes halved with a knife became infected with *Rhizopus* when subjected to relative humidities of 94 to 98 per cent at temperatures of 22.5 to 24° C.

The percentage of infection increases as the humidity is lowered until at a relative humidity of 72 to 84 per cent it is nearly 100.

At relative humidities of 59 to 63 per cent there is decrease in the percentage of infection and at 49 to 52 per cent it is about 20.

At a temperature of 12° C. the relation of humidity to infection is the same as at 22.5 to 25° C. except that the range is narrower.

If potatoes subjected to humidities of 94 per cent at 22.5 to 25° C. are exposed to lower humidities, scarcely any infection occurs, but if potatoes subjected to humidities of 49 to 63 are placed at the same humidities nearly all become infected.

Apparently changes take place at the higher humidities which make the potatoes more resistant to the attack of *Rhizopus*. This resistance seems to be located in the outer cells of the cut surface, because if a thin slice is cut off from the potatoes parallel to the cut surface, these potatoes become infected.

The ascigerous stage of the sweet potato black-rot fungus. JOHN A. ELLIOTT.

The fungus known as *Sphaeronema fimbriatum* has been described as having three forms of asexual spores, two of which are borne on free conidiophores and a third in a long-beaked pycnidium. Examination of embedded and sectioned material has shown that the supposed pycnosporos are ascospores which are apparently liberated within the perithecium by the early disintegration of the ascus. The ascospores are produced by free cell formation within the ascus, each ascus at maturity containing, typically, eight spores having a single well-defined nucleus. The perithecia originate in a hyphal knot, arising, at least in some cases, from the intertwining of branches from two distinct hyphal strands. The taxonomic characters place the fungus in the genus *Ceratostomella* and the specific name *C. fimbriata* com. nov. is therefore proposed for it.

Yellows—Resistant celery: 3rd progress report. G. H. COONS AND RAY NELSON.

Seed production of Golden Self-blanching Celery resistant to yellows (*Fusarium* sp.) was continued on a small scale in 1921 and about a pound of resistant seed is now available for increase. Testing concerned itself with comparing progenies of individual plants for disease resistance. The second generation plants from most isolations possess the same tolerance as the parents and have developed well in infested soil in which commercial seed has failed completely.

Work upon Fusarium—Resistant cabbage in 1922. L. R. JONES, J. C. WALKER AND E. C. TIMS.

The work of the last two summers with disease resistant cabbage has been essentially a continuation of that of preceding years with increasing attention to securing *Fusarium* resistant strains of certain early varieties. In this the developments promise ultimate success although technical difficulties in holding the early varieties over for seed production have slowed up progress. In addition to established strains of the three later varieties (Hollander, All Seasons, and Brunswick), we have promising resistant strains of the earlier varieties (All Head and Glory).

Further experience has shown the uncertainties associated with depending upon ordinary commercial agencies for maintaining a supply of reliable seed, one such agency having distributed seed this last year which proved to have little resistance. We are, therefore, encouraging two distinct methods for meeting this need. The first is that state men or institutions take the responsibility, probably in cooperation with some state or local growers organization, of securing the resistant strains suited to local needs and growing their own seed either directly or by the contract method. Certain localities and organizations are already doing this successfully. Wisconsin cabbage growing districts have done so for several years and the National Kraut Packers Association grew some 4000 pounds of Wisconsin All Seasons under contract last year for its members. As a second method commercial seedsmen are being advised to hold their seed over one year so as to permit of field trial to determine its disease resistant quality before it is offered for sale.

A new blossom-end decay of watermelons caused by an undescribed species of Pythium.
CHARLES DRECHSLER.

At Arlington Farm, Virginia, in 1922, a field disease of watermelons, manifested externally by a dark chocolate-brown or bluish-brown discoloration, that progressed from the blossom region toward the stem end, was decidedly destructive. The rind assumed a watersoaked, candied appearance, while the discolored pulp became flaccid in consistency. The diseased parts were invariably occupied by a fungus closely resembling *Pythium artotrogus* DeBary in size of oogonium and oospore, and in the presence on the oogonial wall of narrowly conical radial processes. However, while in *Pythium artotrogus* fertilization is always accomplished by a "hypogynal" antheridium, in the watermelon parasite it is effected more frequently by multiple branch antheridia, of which from one to four are usually present. The stalk antheridia, moreover, are considerably shorter, their length and diameter being approximately equal. The fungus may occasionally enter the watermelon by wounds or by lesions caused by other fungi. Infection resulted from inoculation with pure cultures under the rind.

The watermelon decay attributable to *Pythium debaryanum* Hesse, which also was abundantly present in the field, is distinguished from the new trouble by light brownish discoloration of the exterior, bleaching of the pulp to pale pink, exfoliation of the epidermis on slight chafing, a peculiar strong marshy odor, and the frequent presence on the moist under-side of extramatrical mycelium.

Seed treatment in the control of pepper diseases. B. B. HIGGINS.

In studying the diseases of pepper which occur in Georgia it has been found that the common leaf spot and fruit rot organisms (*Cercospora capsici* Heald & Wolf, *Bacterium*

sp., *Gloeosporium sp.*, *Colletotrichum sp.*, *Macrosporium sp.*, and *Phoma destructiva* Plow.) may be carried over from year to year on contaminated seed. Since seed contamination is certain where seed are gathered on a commercial scale, efforts are being made to develop a satisfactory method of seed treatment; but the varying response of pepper seed to treatment with disinfectants makes the task difficult. A solution which stimulates germination and subsequent growth at one time may seriously impair germinations of the seed at another. It is hoped that more uniform results may be obtained by treating the fresh seed as they come from the washers. For treating the seed at planting time the best results have been obtained with copper sulphate solution. The seed are soaked in water 6 to 24 hours, drained, soaked 5 minutes in a 1 to 80 copper sulphate solution, dusted with air slacked lime, and planted at once.

An improved formaldehyde tank for the onion drill. A. V. OSMUM AND P. J. ANDERSON.

The only successful method known for controlling onion smut is by the application of formaldehyde in the seed furrow at planting. All investigators have employed essentially the same type of apparatus which has no provision for eliminating variation in rate of application due to reduction of pressure as the column of liquid lowers in the open tank and to speed of operator. To obviate these difficulties a new type of apparatus has been developed at the Massachusetts Agricultural Experiment Station. The chief features of the improved equipment are: (1) a tank made air-tight at the top by a screw-cap, air to replace the liquid being admitted through a stand-pipe attached near the bottom which eliminates variation in rate of flow due to diminishing "head"; (2) changeable discs with graded openings which fit into a union in the delivery pipe regulate the size of the outflow stream to correspond with the rate at which the operator walks. A table based on formulae of application and the time it was found that different operators required to push the drill a certain unit of distance indicates to the operator which of the numbered discs to use. Other improvements of minor importance.

Botrytis rot of the globe artichoke (Cynara scolymus). GEO. K. K. LINK, G. B. RAMSEY AND ALICE A. BAILEY.

Botrytis rot, due to *Botrytis cinerea* (Pers.), causes heavy losses in shipments from California of the globe artichoke.

Although primarily a transit disease it begins in the field where the original contamination and frequently the original infection take place. The fungus was found in the artichoke fields near Half Moon Bay, California. There, favored by heavy fogs and cool weather, the fungus grows and sporulates profusely. Lesions on the growing buds generally are to be ignored in harvesting and packing.

Successful inoculations were obtained by using as inoculum: (1) spore suspension, (2) potato agar cultures, (3) infected bud scales. To get infection by the first method, it was found necessary to saturate the air in the infection chamber with a spray of water once every 24 hours for three to four days. This method probably approximates conditions under which original field and transit infections occur, while the third method is the way in which the disease spreads in transit. The fungus causes very little rot at temperatures above 80° F., and practically none at 0°-2° C., although it grows slightly on agar at 0° C. The rot progresses most rapidly at temperatures between 68 and 75° F., lesions 4-8 mm. in diameter developing within 24 hours after inoculation.

Controlling tobacco wildfire in the seed-bed. P. J. ANDERSON.

On five successive crops of tobacco seedlings in the Experiment Station beds wildfire was reduced to an average of not over one per cent by spraying or dusting the beds with a copper fungicide. The percentage of infection on the controls varied from 20 to 92. Copper lime dust, Bordeaux mixture and various commercial fungicides were all about equally effective. No definite interval between applications can be established, but they must be sufficiently frequent to keep the leaves covered at all times. Dusting is more expensive than spraying but is more rapid and more popular with the growers. Growers who used enough fungicide and applied it frequently enough were successful in keeping the beds clean.

The Sphaerulina leaf spot of clover. E. F. HOPKINS.

This disease which appears not to have been previously reported from America was first observed by the writer in the spring of 1920, on the campus of the University of Missouri. It was later observed in several other places in Missouri, in Illinois and New York. The malady has been noted principally on *Trifolium repens* but *T. hybridum* and *T. pratense* have been found abundantly infected. Inoculation experiments show that *T. pratense perenne* is very susceptible.

Evidence that the disease has been present at Columbia, Missouri, since 1902, was obtained when typical lesions were found on an herbarium specimen, of *T. repens*, collected at that time.

The lesions produced on the leaves are at first very minute, dark spots which usually have at their centers one to several muriform spores. Later the lesions enlarge and are then characterized by a light brown center surrounded by a dark margin. At this stage numerous perithecia appear. A study of these perithecia show the organism to be *Sphaerulina trifolii* E. Rostr. and inoculations made with cultures obtained from young lesions and also from single ascospores proved the organism to be pathogenic. Abundant infections were obtained by exposing healthy plants to leaves bearing perithecia. Lesions found on flower parts indicate that infection of the seed may take place.

The occurrence of zonate eye-spot on various grasses and its mode of extension. CHARLES DRUCKSLER.

During the summer of 1922, *Helminthosporium giganteum* H. & W. was prevalent near Washington, D. C., on a number of grasses besides *Cynodon dactylon* L., on which it was originally described. On this host and on *Phalaris arundinacea* L. the fungus exhibited its greatest destructiveness. *Eleusine indica* (L.) Gaertn., *Bromus inermis* Leyss., *Agropyron repens* (L.) Beauv., *Agropyron elongatum* Host, *Agropyron intermedium* Beauv., and *Lasiagrostis splendens* Kunth, were severely attacked also. Moderate infections occurred on *Agrostis stolonifera* L., *Elymus virginicus* L., and *Leersia virginica* Willd. *Pennisetum japonicum* Trin., *Panicum dichotomiflorum* Michx., *Panicum gattingeri* Nash, *Panicum clandestinum* L., *Panicum anceps* Michx., *Eragrostis major* Host, *Eragrostis pectinacea* (Michx.) Nees, *Muhlenbergia mexicana* (L.) Trin., *Muhlenbergia schreberi* Cmel., *Poa pratensis* L. and *Phleum pratense* L., were spotted to a variable extent when growing with more favorable hosts.

The destructiveness of the fungus is largely dependent on heavy dews or moderately prolonged rains, as the parasite, owing apparently to inability to grow any considerable

distance in the mesophyll seems unable under dry conditions to enlarge the relatively small spots resulting from infections by individual conidia. However, when the affected part is moistened externally, a superficial prostrate mycelium arising from the margins of the dead areas is extended centrifugally and infects the surrounding tissue. This intermittent development continues until the entire leaf blade is involved, the discoloration of the dead foliage presenting a strikingly characteristic zonation.

Sulphur as a fungicide. I. Colloidal sulfur H. C. YOUNG.

Colloidal sulfur was prepared by three different methods, namely, by "The Ralfo," by "The Oden," and by a special method. The toxicity of this form of sulfur was determined by using *Colletotrichum gossypii*, *Glomerella cingulata*, *Gloeosporium venetum*, *Botrytis cinerea*, *Sclerotinia cinerea*, *Phomopsis* sp., *Macrosporium sarcinaeforme*, and *Fusarium oxysporum*. Sulfur in this form in very dilute solutions was found to be very toxic within a narrow limit of hydrogen-ion concentration (about pH 5.8), to all the organisms used. The finer colloidal particles were found to be more toxic, and a general lessening of toxicity was noted as the particles increased in size. Finely divided flowers of sulfur was found to be more toxic than the ordinary flowers of sulfur sold on the market. This toxicity was also influenced by the hydrogen-ion concentration, the point of greatest toxicity being the same as in the case of colloidal sulfur. Changes in the reaction of lime sulfur were also studied. Dilute lime sulfur (1-8) is alkaline beyond the pH range, but changes within a few hours after spraying to pH 6-7.

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THE DISTRIBUTION OF THE ORANGE-RUSTS OF RUBUS

B. O. DODGE

In connection with a line of work primarily devoted to infection experiments with the orange-rusts of *Rubus*, some observations were made on the occurrence of the long-cycled form, *Gymnoconia*, at various localities, in regions considerably south of its hitherto known range. The distribution of the short-cycle rusts has been the subject of much study, some holding that they are more common in the northern regions where the shortness of the season tends to eliminate the accessory spore forms. It is not unlikely that conditions limiting the geographical range of the micro- or lepto-puccinias are entirely different from the factors that determine the distribution of the *Endophyllum*-like short cycled rusts. As pointed out by Arthur (1) the orange-rusts furnish us with a subject, a thorough investigation of which may serve to clear up some of these highly important questions. It is necessary, therefore, to have at hand as far as possible, data regarding the distribution of both rusts, and it is with this in mind that the following notes are submitted. The writer has had the enthusiastic support of Drs. N. E. Stevens and C. L. Shear, who have contributed materially toward furthering this work.

Arthur (1) listed the collections of the orange-rusts in his herbarium either as *Kunkelia nitens* (Schw.) Arth., or as *Gymnoconia interstitialis* (Schl.) Lagh., "more or less arbitrarily," the known distribution of the telial stage being the prime factor in determining whether a given collection of the aecial stage should be listed as the one or the other. The *Gymnoconia* is not shown to occur west of the Mississippi, except in Alaska, otherwise it was supposed to be confined to the region east of the Mississippi and in general north of Ohio, or in the northeastern United States and southeastern Canada. Arthur thinks that there can be no doubt that the northern-most area is occupied by the long-cycled rust and the southern-most area by the short-cycled form. Differences

in the morphological characters of the aeciospores (7) which frequently enable one to predict before germination tests are made which form is at hand, had not been pointed out at that time and germination studies had been made in only a few localities.

Atkinson (3) claims, in effect, that low temperatures inhibit the production of promycelia, so that the nature of the orange-rust would be long-cycled or short-cycled, depending on the temperature at the time of the germination of the aeciospores, a statement easily disproved by a simple experiment.

Kunkel (7) found that the rust on the black raspberry in the vicinity of Washington, D. C., is always long-cycled, and states that the blackberries and dewberries in the same region are attacked only by the short-cycled form. This, if true, would suggest that specific differences in the hosts might also be an important factor in limiting the range of each rust. On the other hand, the long cycled form was found on blackberry much farther south, at Mountain Lake, Virginia, which is at an altitude of about 3,000 feet. This could be cited as evidence that higher altitudes as well as higher latitudes are more favored by the *Gymnoconia*. He says that the aeciospores of the long-cycled form occasionally produce promycelia and do so more frequently when germinated at the low temperatures, 5-10° C., such as one finds in the ordinary ice box. This author did not study the nuclear phenomena in connection with the germinating spores which produced these structures and does not claim that he observed a nuclear fusion, but he assumes that such is the case since he finds what appear to be normal promycelia. Sappin-Trouffy (9) pointed out in a paper overlooked by Kunkel that the aeciospores as well as the uredospores of a number of rusts may germinate rather abnormally. Branched germ tubes with cross walls and supernumerary nuclei are not uncommon in cultures of germinating rust spores. The writer (6) also had described and figured in detail a curious type of germination of aeciospores. As the aeciospore of *Gymnosporangium transformans* germinates the two nuclei may come to lie in a pouch-like enlargement of the germ tube where they divide. The end of the tube grows out and branches more or less irregularly, often forming three or four short divisions into each of which a single nucleus may migrate. A definite cross wall cuts off the forward part of the tube which contains most of the granular cytoplasm from the portion next to the spore. As the culture ages large vacuoles separated by fine lines of cytoplasm help to give these germ tubes the appearance of promycelia. The writer has not studied the germination of the aeciospores of the *Gymno-*

conia very extensively and has not seen any true promycelia although branched germ tubes are not rare. The two orange-rusts are certainly so closely related that one must have been derived from the other, either by a previous fusion of the nuclei in the aeciospore germ tubes, as suggested by the double life history of the *Gymnoconia* observed by Kunkel, or by a delayed fusion followed by the development of a telial stage in a short-cycled form.

Clinton (4) states that both forms occur at New Haven, Connecticut, and Davis (5) now finds the short-cycled form in Wisconsin, where only the *Gymnoconia* was supposed to exist. Just why only *Kunkelia* has been found in Minnesota, while across the river in Wisconsin the *Gymnoconia* is so prevalent, is not clear.

While on a trip to North Carolina in 1921, the telial stage of *Gymnoconia* was found, October 10, on *Rubus argutus* at Ridge Crest, (altitude about 2600 ft.) in the vicinity of Asheville. One of the systemically infected plants was brought to Washington and planted. Aecia matured about May 1, and the spores on germination showed only long, negatively heliotropic germ tubes,—no promycelia.

From April 19–24, 1922, many collections of orange-rust were made at Winston-Salem, North Carolina, in the region where Schweinitz first found *Aecidium nitens*. The long-cycled rust was found on *Rubus occidentalis* back of the Moravian Seminary. All orange-rust found here at this time on blackberry and dewberry proved to be short-cycled.

At Salisbury, North Carolina, April 25, about twenty collections of aecia of a very dark reddish-orange color on blackberry were made. The color of the rust seems to be a very deceptive character, as all found on blackberry here also proved to be short-cycled.

April 25 and 26 some fifty collections of the rusts on blackberries were made in North Carolina between Ridge Crest and Old Fort which is at an altitude of about sixteen hundred feet. Two of the collections on wild blackberry proved to be long-cycled, one obtained at the crest, the other at the base. Four other collections were too young to determine. Black raspberries in the vicinity along the railroad near Old Fort were well infected with the *Gymnoconia* form. On this host aecia were just beginning to mature, while the short-cycled form on blackberries was well out and powdery wherever found. Thinking that perhaps in some localities the *Gymnoconia* might mature a little later, several blackberries having only the pycnial stage at Ridge Crest were marked and on May 17, three weeks later, Rev. J. L. Ray sent rusted leaves from these plants. With one exception they bore the

long-cycled form of the rust. It has also been noted that where both rusts were growing on the same species of dewberry (originally obtained in an infected condition from North Carver, Massachusetts) at Arlington, Virginia, the *Kunkelia* form develops at least a week earlier than the *Gymnoconia*. How far this will prove to be true another year, or in general, of course, must be determined by further observation. It is not improbable, however, that collections made about May 15, at Salem and Bethania would also result in the discovery of the *Gymnoconia* on blackberry in the region covered by Schweinitz.

At Washington further search was made for the *Gymnoconia* on the blackberry and dewberry. On May 24, along the bottom lands of the Patuxent River at Hill's Bridge near Leon, Maryland, it was noticed that the rust on some of the blackberries was a rather dark reddish orange. The two specimens obtained at the time proved to be long-cycled. Three days later sixty collections were made along this roadside and in an abandoned field one mile east. It was easy to determine by the size, shape and color of the spores which form was under observation, a mistake being made in only one case. About half of the infected blackberries along a filling of sand and gravel bore the long-cycled rust. At Pindell cross road the short-cycled rust predominated at one end of the field, fully 50 per cent of the plants being infected. A few rods farther on many plants were infected with the *Gymnoconia*. The black raspberries in the immediate vicinity seemed to be entirely free from the rust. Several infected blackberry plants were transferred to the greenhouse where telia appeared in July on those that survived. An abundance of teliospore material was collected at the Patuxent River locality, July 22 and August 17.

About a dozen infected wild blackberry plants had been obtained near Forestville, Maryland, a few miles from Washington, on April 11. It took some time for them to recover after being potted, but by May 31 most of them had mature aecia on the shoots that had grown out since transplanting some light orange in color, others a darker reddish orange. Germination tests proved that one plant, No. 291, had the long-cycled rust; two others, Nos. 292 and 296, had a rust whose spores were large, spherical, of uniform size, and a dark reddish-orange color. These spores produced at once negatively heliotropic germ tubes, which after growing out vigorously for some distance became very regularly perfect promycelia bearing sporidia. Several other plants from this place developed the more typical *Kunkelia*. Telia were found at Forestville, Maryland, July 22, where these plants had originally been obtained.

Several collections of orange-rust were made at Bethlehem, Pennsylvania, June 10 to 12. A number of plants of *Rubus occidentalis* bore a light orange colored rust which proved to be long-cycled. A number of wild blackberries also bore the Gymnoconia. On the hill above Lehigh University, two blackberries bearing the long-cycled rust grew near ten others infected with the short cycled form. These could be distinguished by the color of the rust, although germination tests of the spores are always necessary to prove each case.

Five specimens of rust were collected on blackberry by Dr. Shear at Dover, Ohio, June 17, 1922. The two on the cultivated variety, Eldorado, proved to be the Gymnoconia, while one each on a wild blackberry, *Rubus allegheniensis*, and a cultivated variety, Mercereau (?), were short cycled. April 25, a specimen of Kunkelia was received from Oklee, Minnesota, on cultivated blackberry. Oklee is in 48° north latitude where the winters are notably very severe.

On May 12, 1921, Dr. Stevens sent in several infected plants from Woodstock, New York. In each case tests proved that the rust on blackberries and dewberries was short-cycled, but on the black raspberry the rust was long-cycled. The same region was visited on May 30, 1922. One collection on blackberry at 2,000 feet was long-cycled, while one made on wild dewberry at 1,000 feet was short-cycled. The rust on *Rubus occidentalis* at both of these altitudes was long-cycled. Random collections made at North Carver, Mass., May 27, 1921, showed that both forms grow here on blackberry side by side, five specimens being Kunkelia and five Gymnoconia. Several collections made July 8, the same year, from this locality proved to be long-cycled. The Gymnoconia has been found in Maine at Wells, North Livermore, Leeds Junction, Lewiston and Phillips on *Rubus canadensis* and other wild blackberries and dewberries. We have not found Kunkelia as yet in this state.

A great many germination tests have been made of aeciospores from *R. occidentalis* within a radius of twenty miles of Washington, D. C., without finding the Kunkelia on this host.

Experiments to be described elsewhere show that the following cultivated varieties of blackberries are susceptible to infection by the Kunkelia at Washington: Iceberg, Crystal White, Mercereau, Crandall, Kittatinny Loganberry, Ward, Taylor and Eldorado (?). Telia of the Gymnoconia have been collected on leaves of the Ward, Loganberry, Eldorado, and Kittatinny, Mercereau, Taylor and the mountain blackberry, *R. canadensis*, in the same region.

The following table may be considered in the nature of a supplement to Kunkel's list of collections (7), the aeciospores of which were tested by germination. Where more than one collection was made at a given locality on a certain date the number tested is sometimes shown in parenthesis. While the color of the aecium is a character frequently helpful in predicting how the spores will germinate, it is not a constant feature and it is not mentioned in our table. For example, at Bethania, N. C., April 23, 1922, short cycled aecia on blackberry were a very dark reddish- or brownish-orange color; at Old Fort, N. C., April 25, the color of the same form varied from light lemon-yellow to deep xanthine orange, regardless of the state of maturity of the aecia.

TABLE 1

Place and time of collection, host and manner of germination of the aeciospores of various collections of orange rusts

Place of collection	Time of collection	Host	Manner of germination ¹
Wells, Me.	July 10, 1922	Wild blackberry	Germ tubes
Arlington, Va. (From Phillips, Me.)	May 1-10, 1922	Wild dewberry (14)	" "
Phillips, Me.	June 27, 1921	<i>R. canadensis</i>	" "
Wells, Maine	July 5, "	Wild dewberry (3)	" "
North Livermore, Me.	June 27, "	Wild blackberry (2)	" "
Portland Farms, Me.	July 5, "	" " (3)	" "
Leeds Jct., Maine	" 1, "	Wild dewberry (6)	" "
Lewiston, Me.	June 28, "	" " (7)	" "
Phillips, Me.	" 27, "	" "	" "
Phillips, Me.	July 8, "	" "	" "
N. Woodstock, N. H.	June 20, "	" " (4)	" "
N. Woodstock, N. H.	" 21, "	" " (3)	" "
Profile Lake, N. H.	" 24, "	Wild blackberry & wild dewberry (12)	" "
Bartlett, N. H.	July 11, 1922	Wild blackberry	" "
Franklin, N. H.	June 21, 1921	Wild dewberry	" "
Bartlett, N. H.	" 23, "	<i>R. canadensis</i> (7)	" "
Beamis, N. H.	" 21, "	<i>R. canadensis</i>	" "
Crawford Notch, N. H.	" 20, "	Wild blackberry (4)	" "
Woodstock, N. Y.	May 12, "	" " (8)	Promycelia
Woodstock, N. Y.	" 12, "	<i>R. occidentalis</i>	Germ tubes
Woodstock, N. Y. (1,000 ft. alt.)	" 31, 1922	Wild dewberry	Promycelia

¹ Miss Ruth Colvin made many of the germination tests here reported.

TABLE 1 (*Continued*).

Place of collection	Time of collection	Host	Manner of germination
Woodstock, N. Y. (1,000 ft. alt.)	May 31, 1922	<i>R. occidentalis</i>	Germ tubes
Woodstock, N. Y. (2,000 ft. alt.)	" 31, "	Wild blackberry	" "
Woodstock, N. Y. (2,000 ft. alt.)	" 30, "	<i>R. occidentalis</i>	" "
Marlboro, N. Y.	May 18, 1921	Wild Rubus spp. (5)	Promycelia
Pine Bush, N. Y.	May 28, 1922	Wild blackberry	Germ tubes
Bull Hill, N. Y.	May 28, "	Wild blackberry	" "
Bull Hill, N. Y.	May 28, "	Cultivated blackberry	Promycelia
Brooklyn, N. Y.	July 1, 1920	Wild blackberry	"
Reading, Mass.	" 3, "	Cultivated blackberry	"
North Carver, Mass.	May 27, 1921	Wild dewberry (3)	Germ tubes
North Carver, Mass.	June 6, 1921	<i>R. occidentalis</i> (3)	" "
North Carver, Mass.	" 6, "	Wild blackberry (2)	Promycelia
North Carver, Mass.	May 27, 1921	Wild dewberry (5)	"
Salisbury, Mass.	July 10, 1922	Wild blackberry (2)	Germ tubes
Pittsfield, Mass.	June 2, 1921	<i>R. occidentalis</i>	" "
Canaan, Conn.	May 20, "	Black raspberry (5)	" "
Hammononton, N. J.	June 5, 1922	Wild blackberry (2)	Promycelia
Cologne, N. J.	" 6, "	" " (3)	Promycelia
Bunola, Penn.	May 16, 1920	Cultivated blackberry	"
Bethlehem, Penn.	June 8, 1922	Wild blackberry (10)	"
Bethlehem, Penn.	" 8, "	" " (5)	Germ tubes
Bethlehem, Penn.	" 8, "	<i>R. occidentalis</i> (4)	" "
Bethlehem, Penn.	" 8, "	Wild dewberry (2)	Promycelia
Bell, Maryland (Holly Hill, Fla.)	May 15, "	Dewberry × blackberry	"
Bell, Md.	" " "	Ward blackberry	"
Bell, Md.	" " "	Mammoth dewberry (seedling)	"
Bell, Md.	" 15, "	<i>R. tetrahizans</i>	Promycelia
Leon, Md.	" 24, "	Wild blackberry (2)	Germ tubes
Leon, Md.	" 24, "	<i>R. cuneifolius</i>	Promycelia
Leon, Md.	" 27, "	Wild blackberry (31)	Germ tubes
Leon, Md.	" 27, "	" " (29)	Promycelia
Forestville, Md.	" 27, "	" " (4)	Germ tubes
Forestville, Md.	" 27, "	" " (10)	Promycelia
Forestville, Md. (greenhouse plant)	" 31, "	" " (1)	Germ tubes
Forestville, Md. (greenhouse plants)	" 31, "	" " (7)	Promycelia

TABLE 1 (Continued).

Place of collection	Time of collection	Host	Manner of germination
Pasadena, Md.	May 24, 1922	<i>R. hispidus</i>	Promycelia
Pasadena, Md.	" 24, "	<i>R. cuneifolius</i>	"
Woodward, Md.	" 30, "	Wild blackberry (5)	"
Fort Myer, Va.	April , "	Cultivated blackberry	Germ tubes
Arlington, Va.	April , "	Loganberry	Promycelia
Bedford, Va. (3850 ft.)	July 2, "	Wild blackberry (2)	"
Vienna, Va.	May 30, 1921	Wild blackberry (2)	"
Vienna, Va.	" 10, "	<i>R. occidentalis</i> (4)	Germ tubes
Prospect Hill, Va.	" 22, "	<i>R. cuneifolius</i> (5)	Promycelia
Prospect Hill, Va.	" 22, "	Wild dewberry (3)	Promycelia
Old Fort, N. C. (1650 ft. alt.)	April 25, 1922	<i>R. canadensis</i> × <i>R. nigrobaccus</i>	Germ tubes
Ridgecrest, N. C.	" 26, "	<i>R. nigrobaccus</i> (?)	Germ tubes
Ridgecrest, N. C.	" 26, "	<i>R. nigrobaccus</i> (?) (30)	Promycelia
Ridgecrest, N. C.	May 17, "	<i>R. nigrobaccus</i> (?) (5)	Germ tubes
Ridgecrest, N. C.	" 17, "	<i>R. nigrobaccus</i> (?)	Promycelia
Salisbury, N. C.	April 24, 1922	Wild black raspberry (2)	Germ tubes
Salisbury, N. C.	" 24, "	Wild blackberry (20)	Promycelia
Old Fort, N. C.	" 25, "	Wild blackberry (many)	"
Winston-Salem, N. C.	" 20, "	Wild blackberry and dewberry (24)	"
Winston-Salem, N. C.	" 21, "	<i>R. occidentalis</i> (3)	Germ tubes
Bethania, N. C.	" 23, "	<i>R. argutus</i> (10)	Promycelia
Pilot Knob, N. C.	" 23, "	Wild blackberry (5)	"
Pilot Knob, N. C.	" 23, "	Wild dewberry (4)	"
Winston-Salem, N. C.	" 22, "	<i>R. Enslenii</i> (10)	"
Winston-Salem, N. C.	" 22, "	<i>R. trivialis</i> (3)	"
Cameron, N. C.	" 17, "	Wild blackberry (11)	"
Charleston, S. C.	Mar. 21, "	Cultivated blackberry	"
Lotte, Fla.	Feb. 20, 1921	" "	"
Oklee, Minn.	June 23, 1922	Cultivated blackberry	"
South Haven, Mich.	" 22, "	<i>R. occidentalis</i>	Germ tubes
Rockford, Ill.	" 17, "	Cultivated blackberry	Promycelia
Dover, O.	" 19, "	Wild blackberry	"
Dover, O.	" 19, "	Eldorado blackberry (2)	Germ tubes
Dover, O.	" 19, "	<i>R. alleghaniensis</i>	Promycelia
Dover, O.	" 19, "	Mercereau? blackberry	"
St. Louis, Mo.	May 27, "	Cultivated blackberry	"
Fort Worth, Texas.	April. 2, 1921	Cultivated blackberry	"

Considerable uncertainty exists regarding the particular species of *Rubus* upon which Schweinitz first found what he called *Aecidium*

nitens. In his first published description of the rust (10) he cites *Rubus strigosus* as the host, and it is so cited in his first manuscript. In his later publication (11) he simply states that the rust is found on *Rubus*.

Arthur (1, p. 504) describes the new genus *Kunkelia*, citing Schweinitz's Salem collection as the type (Cf. Arth. & Bisby, 1918, p. 230). In the former paper Arthur makes the new combination as follows:—" *Kunkelia nitens* (Schwein.), comb. nov.—*Aecidium nitens* Schwein. Schrift. Nat. Gesell. Leipzig 1: 69 1822 (type on *Rubus* '*strigosus*' error for *R. Enslenii*, Salem, N. C.)." The discussion of their studies of the collections of the orange-rust in the Schweinitz Herbarium at the Philadelphia Academy by Arthur and Bisby (2) is not altogether clear. Schweinitz cites *R. strigosus* as the host in his first published account instead of the last, and the long-cycled rust has certainly been reported several times since 1822 on *R. strigosus*. It would be indeed a very small matter on just what particular species of *Rubus* Schweinitz first found *Aecidium nitens* if there were only one orange-rust on *Rubus* or even only one at Salem, N. C. But if both long- and short-cycled forms are today found at Salem, N. C., and much farther south, it is a question whether Schweinitz may not after all have first found the long-cycled form, not on the red raspberry *Rubus strigosus*, but on the black raspberry, *R. occidentalis*, which today can be found there infected with the *Gymnoconia*.

Arthur and Bisby (2) state that the orange rust in the Schweinitz Herbarium at Philadelphia is represented by five leaves in a mounted packet and by many leaves and leaflets in the original packet, which is labeled inside "*Aecidium nitens* in Rubo villosa Salem Bethl Neujork." These authors state that it was the custom of Schweinitz to preserve but the one original collection to represent each species. "It is quite evident from its appearance that the ample material of the present species was all gathered at one time and that it is all, or nearly all, from one plant as it is very uniform. A part of the material has been seen by Dr. P. A. Rydberg . . . and he states that the host cannot possibly be *R. strigosus*, but that it may be *R. procumbens* Muhl., or more likely, its southern representative *R. Enslenii* Walt., both of which usually passed under the name '*R. villosus*, a century ago.'"

Through the kindness of Mr. Eugene A. Rau of Bethlehem, Penn., Dr. Shear and the writer were able to examine an unpublished manuscript of Schweinitz entitled "*Flora Salemitana sistens Plantas et Vegetabilia omnia huc usque in agro Salemitano Carolinae Septentrionalis observata Radio triginta millium ex Carolinae Septentrionalis Comitatus Stokes, Surry, Guilford, Rockingham Et Rowan. Autore L. D. v. Schweinitz*

1821 Aprili fine. In margine additas envenies Plantas quas ex Bethlehem Pennsylvania loco natali observatum." The following extract may serve to clear up some of the uncertainty as to Schweinitz's knowledge of species of *Rubus*.

Page	72	271	Rubus (Salem)
common	738		1. occidentalis our common raspberry.
common	739		2. villosus this appears to suit best our common blackberry.
common	740		3. trivialis the common dewberry.
rare	741		4. flagellaris rarer v. c. at Quaker.
not rare	742		5. obovalis at the double branches
	hispidus Easton		
	odoratus Easton, Bethl. Lehigh Mts.		
	strigosus, Blue Mts.		
	cunefolius, Hope (N. J.)		

This list indicates clearly that Schweinitz did not use the name *villosus* to include the dewberry as suggested by Rydberg in the above quotation. The specimen in the Schweinitz Herbarium at Philadelphia labeled *R. villosus* is a true blackberry and has been labeled *R. allegheniensis* by Blanchard and has been placed in the cover with *R. argutus*.

Dr. Shear, who is familiar with the work of Schweinitz and his methods, has also examined the collections of orange rust in the Schweinitz Herbarium at Philadelphia, and has furnished the following memorandum which throws more light on the question of the type of *Aecidium nitens*.

"Schweinitz probably used the name *Rubus strigosus* for the host upon which his first specimens of the orange rust were collected on account of a misunderstanding of the application of this name which had only a short time before (8, p. 296) been published by Michaux. A list of the botanical works in Schweinitz's library recently obtained by the writer through the kindness of Mr. Eugene A. Rau of Bethlehem, Pennsylvania, shows that this was one of the books that he used and the one from which he probably derived this name and the description. In the interval between 1817, when Schweinitz finished the manuscript of his *Fungi of North Carolina*, and 1821, when he prepared the above list of Rubi, he apparently discovered that he had applied the name *strigosus* erroneously to plants growing about Salem and therefore abandoned it as it does not occur in this list. Whether in this case, which is the only one we

can find where he used this name for specimens of *Rubus* from North Carolina, either as the host of a fungus or as a member of the flora, he applied it to *Rubus occidentalis* or to *R. villosus* of the older authors cannot perhaps be determined with certainty at present, as part at least of his original specimen of *Accidium nitens* was sent to Schwaegrishen with his manuscript in 1817, and if extant its present location is unknown. In any case it is not at all probable that the original host was *R. strigosus*, as this species does not occur about Salem now and probably never did. It seems much more likely that Schweinitz applied the name erroneously to *R. occidentalis* rather than *R. villosus* or *R. procumbens* as Michaux's brief description states that the leaves of *strigosus* are white tomentose underneath and this would not apply to any of the blackberries or dewberries known to occur in Salem or vicinity.

An examination of such of Schweinitz's specimens of the rust as are available appears to throw some light on this question. While it does not seem possible for a *Rubus* expert to tell with much certainty the species of *Rubus* to which a leaf completely covered with aecia of the orange-rust belongs, yet it is perfectly practicable after considerable study and comparison of rusted leaves in the field to tell whether any particular specimen came from a raspberry as compared with a blackberry or dewberry. Rusted raspberry leaves are a different color and texture from rusted blackberry or dewberry leaves. The rusted raspberry leaves are thinner, more fragile and paler green color than the others.

Schweinitz's mycological collections when transferred to the Philadelphia Academy of Natural Science were preserved in small paper packets which were placed in large packets and tied in quarto board covers. Later, in 1854-1857 Michener removed, in some cases part and in some cases all, of the specimens from the original packets and glued them to squares of paper and mounted these in folio volumes. In the case of *Accidium nitens* only a part of the material in Schweinitz's original packet was removed and mounted by Michener. The original packet bears the following data in Schweinitz's hand-writing:

"Accidium nitens
in Rubo villosa
Salem Bethl. New York."

"There is evidence here, as in many other cases of Schweinitz's specimens which we have examined, that one of the leaves had originally been glued to the sheet in which the rusted leaves are now wrapped. As has already been pointed out (*Endothia Parasitica* and Related Species,

Bulletin 380, U. S. D. A., page 7, 1917) the evidence shows that Schweinitz at one time mounted his specimens of fungi by gluing them to sheets of paper and writing the labels on the sheets beneath the specimens. Later he changed his plan, removed the glued specimens from the sheets, cut up the paper on which a specimen had been mounted, folded it into a packet and placed the original specimen inside; and in many cases at least also added later other specimens from the same or different localities. On this particular packet, apparently later and with different ink, was written *Caecoma luminatum*, the name which he used in his later publication in 1832. In the original packet, as well as the mounted specimen, there are leaflets of two quite different species, one of them possessing the characters of rusted *R. occidentalis* leaves and the other clearly belonging to the blackberry-dewberry type. A leaf of the *occidentalis* type seems to be the one which was originally glued to the herbarium sheet, and if we were justified in assuming that this is a part of the type or the same gathering as that Schweinitz sent Schwaegrishen with the manuscript of his Syn. Fun. Car. Sup. there would be strong reason for believing that the type of the orange-rust was on *R. occidentalis* and, as at present we have found only the long-cycled rust on this host, it might be concluded that Schweinitz's name should be applied to the long-cycled rust. As, however, the evidence is not entirely conclusive, it is probably better to follow the precedent established by Arthur and apply Schweinitz's name to the short cycled rust."

In the case of rust collected on *Rubus* in April, 1922, at Salem, N. C., it was possible in several instances to obtain specimens of only partially rusted plants or of infected plants which were root sprouts from those free from rust and then in blossom. We are indebted to Dr. P. A. Rydberg for identifying the hosts as far as possible. It appears that today the *Gymnoconia* occurs on *R. occidentalis* at Salem, and that the *Kunkelia* is to be found there on *R. argutus*, *R. floridus*, *R. Boyntoni*, *R. trivialis* and *R. Enslenii*. We have no specimens of rust on Salem plants identified by Dr. Rydberg as *R. frondosus*, *R. betulifolius*, and *R. Baileyanus*. The rust is more common on blackberries than on dewberries. If the rust on the black raspberry is always long-cycled as Kunkel found it to be in the vicinity of Washington, D. C., the distribution of the *Gymnoconia* probably coincides with the range of the host, *R. occidentalis*.

There is still much to be desired in the way of knowledge regarding the distribution of these rusts. Careful germination tests must be made in every case as there is no known constant morphological character by which the rusts can be distinguished. It would be especially instructive

to determine just how far north in the New England States the *Kunkelia* grows. Further investigation of the rust on the cultivated black raspberries west of the Mississippi should be made, as the *Gymnoconia* is more likely to be found on this host.

As one travels from Florida through the coast states to Maine, he is struck by the change from one orange rust to the other, the short-cycled clearly predominating in the South and the long-cycled in the North. In strong contrast, however, in the states along the western bank of the Mississippi, from Texas to Canada, the short-cycled, according to Arthur, alone occurs.

Many specimens of orange rust have been gathered in the White Mountains of New England during the latter part of June and the first week in July. We were therefore much surprised to find on July 10, 1922, that the aecial stage had almost entirely disappeared at Wells, Maine, and at Bartlett, New Hampshire. Specimens obtained showed green leaves covered with maturing spermogonia interspersed with telia. There were no signs of aecial scars on these leaves but such scars could generally be found on some of the fallen leaves at the base of infected plants. Did telia follow spermogonia so quickly on infected leaves as to entirely prevent the formation of aecia? It is presumed that such telia resulted from infection by aeciospores, but should cell fusions sometimes occur and these result in the development of telia we can see how a short-cycled *Puccinia* might arise.

What are the facts regarding the *Gymnoconia* on *R. strigosus* or *Kunkelia* on *R. occidentalis*? In view of the number of species of *Rubus* known to be susceptible to attack by both rusts we should expect that *R. occidentalis* would harbor the *Kunkelia* as sometimes reported. If *Kunkelia* does occur on this host, it must be only occasionally, as every collection, so far tested by the writer, has turned out to be the *Gymnoconia*. These rusts attack plants with all degrees of severity, frequently the second year the plants are so completely transformed that it is almost impossible to tell one infected species of blackberry or dewberry from another. This being the case, the specific names assigned to the hosts in no small percentage of herbarium specimens should not be considered as altogether final, especially where the collector simply endeavors to assign as nearly as possible a specific name to the host as the result of a general demand for such a name on the label.

The host is frequently given as *R. strigosus* in herbarium specimens of these rusts when it is clear that the host is a blackberry. There are seven packets of orange-rust in the Pathological Collections of the United

States Department of Agriculture, labeled to indicate that the host is *R. strigosus*. In every case except one the host is not a red raspberry. Nos. 277, 278, Ellis, N. Am. Fungi, are among this list. In No. 1014, Herbarium H. S. Jackson, the host which here bears only the telial stage is certainly either *R. strigosus* or *R. neglectus*, which is now recognized as a hybrid, the parents being *R. occidentalis* and *R. strigosus*. Specimens sent to the writer also proved that *Rubus* hosts are often confused. Where the host has been identified as red raspberry by the collector it is usually a blackberry.

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A BACTERIAL DISEASE OF BROME-GRASS

CHARLES S. REDDY AND JAMES GODKIN¹

WITH PLATES I AND II

INTRODUCTION

In 1916, Professor L. R. Waldron, of the North Dakota Agricultural Experiment Station, called the attention of the senior author, then stationed at that place, to an apparently new disease of brome-grass (*Bromus inermis* Leyss.). The symptoms suggested that it was caused by bacteria, and subsequent isolations tended to confirm this. No fungi were found, and bacteria were readily isolated by methods herein described. On account of pressure of other duties, no inoculations were tried at that time. In the fall of 1919, the authors took up the problem. The investigations, extending over a period of three years, have been conducted at the University of Wisconsin and at Bloomington, Illinois. Numerous isolations and spray inoculations in the greenhouse have proved that the disease is caused by a colorless bacterium pathogenic to brome grass.

DESCRIPTION

In the early stages the disease appears on the leaves of *Bromus inermis* as light olive green, circular to elliptical water-soaked areas with light brown centers. Later the spots become dark chocolate or purplish brown and appear almost black. Old spots are usually linear, often coalescing to destroy the entire leaf surface of the infected plants. In early stages the spots are characterized by faded green to yellowish-green halos, which become more definite in the older spots.

In severely attacked plants, the upper nodes sometimes are killed by secondary infections of the organism. In these plants the panicles wither and die, producing symptoms similar to frost injury.

The seed may become infected by coming into contact with lesions on the upper leaves at the time the panicle emerges from the boot, or later the panicle may be blown by the winds so as to come into contact with leaf lesions. Also, splashing rains cause the disease to spread to the heads, as well as to other parts of the plant.

¹ These investigations were conducted at the University of Wisconsin during 1919, 1920, and 1921 under the direction of Professor L. R. Jones.

OCCURRENCE AND HOST RANGE

Each year from 1917 to 1921, the disease has been collected in the vicinity of Fargo, North Dakota, on *Bromus inermis*. In April, 1921, the disease, exhibiting its characteristic symptoms, was found on *B. inermis* at Madison, Wisconsin. In 1915, the senior author collected a bacterial disease on quack grass (*Agropyron repens* (L.) Beauv.) which exhibited symptoms similar to the brome-grass disease. A second collection on quack grass was made in company with A. G. Johnson in the fall of 1919 from which a colorless bacterium was isolated and proved pathogenic on *B. inermis*. As stated later, the brome-grass organism was found to be pathogenic on quack grass. Although no exhaustive comparison has been made between the two organisms, it is probable that they are identical. A spot on yellow foxtail is very common in the vicinity of Bloomington, Illinois. Since a bacterial disease had been reported previously on this host by Rosen (4), isolations were attempted. No bacterium could be found associated with these lesions, and later, inoculations with the brome-grass organism on yellow foxtail gave negative results. Natural infections on other hosts have not been observed although artificial inoculations have materially increased the host range.

ISOLATION OF THE ORGANISM

The blackened areas of the lesions were found to contain the bacteria in large numbers. The method most commonly used in making isolations was first to wash an infected leaf in clean, running water and then to cut pieces, about the size of the head of a pin, from the blackened areas of the lesions. Each of these pieces then was placed in a drop of sterile water previously placed in the center of a sterile petri dish by means of a flamed 4-mm. platinum loop. The tissue pieces were then mashed with a sterile instrument. When thinly seeded plates were desired, a small loop from each of the drops containing mashed tissue was transferred to each of another set of sterile petri dishes. Plain nutrient- or potato-dextrose agar then was poured into each of these in the usual manner and the dishes tipped back and forth in the usual way to distribute the bacteria uniformly. Colonies appeared in 36 to 48 hours and within three days after plating, streaks from single colonies were made on agar slants.

Isolations were made from both fresh and dried material. Isolations, from which the first artificial infections were obtained, were made from herbarium material, six months old, collected by the senior author

while at the North Dakota Agricultural Experiment Station. Subsequent isolations of the brome-grass organism have been made from this material up to 33 months after collection.

INOCULATION EXPERIMENTS

On *Bromus inermis*

The pathogenicity of each isolation was determined by inoculating *Bromus inermis*. At first 2 methods were used, wounding and spraying. The latter method gave more typical results, hence it alone was used in most of the later experiments. This method was essentially as follows: Sterile water was poured into tubes containing 2- to 5-day old agar streak cultures of the organism, and the bacterial growth was then allowed to diffuse into it. This suspension of the organism was applied by means of atomizers to the plants of various ages grown in pots. These were placed in damp chambers or covered with shaded bell jars for about 48 hours. The ends of the first leaves are very susceptible to infection and in 2 to 4 days turn black and wither. The more typical lesions appear in from 3 to 9 days on any parts of the leaves as small water-soaked infection points each with the indication of a halo about it. The water-soaked spots enlarge, gradually become darker until they are purplish black, and the halo, although usually not extending itself very far, becomes more distinct.

On Other Species of *Bromus*

A number of species of *Bromus* were grown in 4-inch pots in the greenhouse and, by means of atomizers, were inoculated without wounding when the plants were about 4 inches high. Both the brome-grass organism and the halo-blight organism (*Bacterium coronafaciens* Elliott) from oats were used. The results are presented in table 1. The plants in two pots of each species held as controls remained free of the disease. The degree of infection for each species in each experiment is indicated in the table as follows: 0 = no infection; 1 = slight infection; 2 = moderate infection; 3 = heavy infection.

All the species of *Bromus* inoculated were more or less susceptible to infection by the brome-grass organism. The resulting lesions in all cases, except on the five species noted below, were similar to those previously described for *Bromus inermis*. On *Bromus brizaeformis* Fisch. and Mey., *Bromus japonicus* Thunb., *Bromus molliformis* Lloyd, *Bromus tectorum* L., and *Bromus tectorum nudus* Klett and Richter, the centers of infection did not become black but were small, sunken, and

light gray with somewhat larger halos, resembling to a marked degree the halo-blight of oats. Of the species of *Bromus* inoculated with *Bacterium coronafaciens*, lesions developed on *Bromus carinatus* Hook. and Arn. only. These were typical "halo-blight" lesions with dead centers and large halos.

TABLE 1

Results of inoculations of species of Bromus with the brome-grass organism and Bacterium coronafaciens

Host	Brome-grass organism			<i>Bacterium coronafaciens</i>
	Expt. No. 1	Expt. No. 2	Expt. No. 3	Expt. No. 4
<i>B. arenarius</i> Labill.....	1			0
<i>B. brizaeformis</i> Fisch. and Mey	2			0
<i>B. carinatus</i> Hook. and Arn	2	1	1	1
<i>B. carinatus linearis</i> Shear	2			0
<i>B. commutatus</i> Schrad	1			0
<i>B. erectus</i> Huds	2			0
<i>B. hordeaceus</i> L.....	2	2		0
<i>B. hordeaceus</i> L. (Madison)	2	2	2	
<i>B. inermis</i> Leyss. (Arlington)	1	1		0
<i>B. inermis</i> Leyss. (Madison)	3	3	3	0
<i>B. japonicus</i> Thunb	1	1		0
<i>B. lanuginosus</i> Porr	1			0
<i>B. marginatus</i> Nees	1			0
<i>B. molliformis</i> Lloyd	1			0
<i>B. mollis</i> L.....	1			0
<i>B. pratensis</i> Lam	2			0
<i>B. richardsonii</i> Link.....	3	3		0
<i>B. rubens</i> L.....	2			0
<i>B. secalinus</i> L.....	2			0
<i>B. secalinus</i> L. (Madison)	2	2	2	
<i>B. sitchensis</i> Bongard	1			0
<i>B. sterilis</i> L.....	2			0
<i>B. tectorum</i> L.....	2			0
<i>B. tectorum</i> L. (Madison)	2	2	2	
<i>B. tectorum nudus</i> Klett and Richt.....	1			0
<i>B. unioloides</i> (Willd.) H. B. K.....	3	3		0
<i>B. villosus</i> Forsk ...	2			0

On Other Grasses

In six series of artificial inoculations made in the usual way the pathogenicity of the brome grass organism was tested on the common cereals and certain grasses. In each series brome-grass (*Bromus inermis*) be-

came moderately to heavily infected, quack grass (*Agropyron repens*) became slightly to moderately infected, and in two series oats became slightly infected. In all the series the results were negative on wheat (*Triticum aestivum*, L.), barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), bluegrass (*Poa pratensis* L.), red top (*Agrostis palustris* Huds.), orchard grass (*Dactylis glomerata* L.), corn (*Zea mays* L.), timothy (*Phleum pratense* L.), yellow foxtail (*Chaetochloa lutescens* [Weigel] Stuntz), and barnyard grass (*Echinochloa crus-galli* [L.] Beauv.). In all, *Bromus inermis* has been inoculated approximately 24 different times and always with positive results, and the organism reisolated repeatedly.

In addition to the experiments referred to above, several parallel cross inoculations were made on brome-grass and oats, using both the brome-grass organism and the halo blight (*Bacterium coronafaciens*) from oats. Both organisms infected oats, but the lesions were not identical. Those caused by the brome-grass organism had a more water-soaked appearance and narrower halos than those caused by *B. coronafaciens*. *Bacterium coronafaciens* failed to infect brome-grass in these inoculation experiments, while, at the same time, good infections were secured with the brome-grass organism. As will be seen later, it is mainly on the basis of these cross inoculation experiments that the authors have felt justified in considering the brome-grass organism a variety of *B. coronafaciens* rather than identical with it.

CULTURAL CHARACTERS OF THE ORGANISM

This organism resembles *Bacterium coronafaciens* (3) except for minor differences. Hence, it seems necessary to compare them in only a few pertinent points and to present any additional studies made upon the brome-grass organism.

Bacterium coronafaciens Elliott from oats

Brome-grass organism

- | | |
|--|--|
| 1. Average size 0.65 by 2.3 μ when stained with Ribbert's capsule stain, gentian violet, and carbol fuchsin. | 1. Average size 0.72 by 1.92 μ when stained by aqueous gentian violet. |
| 2. Thermal death point 47° to 48° C. Growth at 0° C. | 2. Thermal death point 50° to 51° C. No growth at 0° C. |
| 3. Killed by desiccation on cover glasses within 24 hours. | 3. Remained alive in dried condition on cover glasses up to 198 hours. |
| 4. Facultative anaerobe—growth in closed arms of tubes containing saccharose, glycerine, mannit, levulose, and dextrose. | 4. Strict aerobe. |

Bacterium coronafaciens Elliott
from oats

5. Good growth in 0.1 per cent citric, tartaric, and malic acid.
6. No clouding in neutral beef-peptone bouillon containing more than 2 per cent sodium chlorid.

Brome grass organism

5. No growth in 0.1 per cent citric, tartaric, and malic acid.
6. Clouding in neutral beef-peptone bouillon up to and including 7 per cent sodium chlorid.

Excluding infection experiments, the above statements present the differences in the morphology and physiology of the brome-grass organism compared with the morphologic and physiologic characteristics of *Bacterium coronafaciens* as given by Miss Elliott.

Realizing that these differences might be due to one or more of a number of factors, a culture of the oat organism was obtained from Miss Elliott and some of the experiments hitherto showing unlikeness of the two were repeated using both organisms side by side.

When the bacteria from 24-hour old cultures on plain nutrient agar were stained with aqueous gentian violet, the brome-grass organism averaged 0.72 by 1.92 μ , and the oats organism averaged 0.69 by 1.95 μ . The brome-grass organism from 7-hour old cultures on the same medium averaged 0.81 by 2.25 μ . All of these averages are measurements of 100 organisms in each case. Taking into consideration the possible variations in size, the two organisms can be considered identical morphologically.

Since it has been shown by Wolf and Foster (6) that, with plant parasites, variation in the reaction of the bouillon may cause the thermal death point to vary as much as 4° to 6° C., and since it is likely that other factors have as much effect, it was thought unnecessary to repeat the thermal death point experiment because the thermal death points already checked within 3 degrees.

Desiccation on cover glasses was not considered an important point of difference. The experiment was performed twice, using the brome-grass organism and growth occurred up to 175, and 198 hours respectively.

Growth occurred in shake cultures of plain nutrient agar at or near the surface only. No growth occurred in the lower end of stab cultures of plain nutrient agar or gelatin. No growth occurred in Roux anaerobic tubes filled with inoculated melted agar except at the ends of a few in which the agar did not completely fill the tubes. No increase in growth occurred in cultures placed in Novy anaerobic jars. At first growth was not consistent in the closed arm of fermentation tubes containing the various carbon compounds which was doubtless due to daily examination which necessitated handling the tubes. In this process a small

amount of the copious growth in the open arm was probably transferred to the closed arm and upon distribution gave the closed arm the appearance of being slightly clouded. When both organisms were used in a complete fermentation experiment and half of each set was left undisturbed for 17 days, there was no growth in the closed arms of the undisturbed tubes. This was true in the case of those inoculated with *Bacterium coronafaciens* as well as in the case of those inoculated with the brome-grass organism. In the tubes that were handled each day, part of them showed clouding in the closed arms. These results indicate that, although the two organisms are identical in this characteristic, they are obligate aerobes rather than facultative anaerobes.

Both organisms were grown in bouillon (pH = 6.8) containing various percentages of sodium chlorid up to 8. Both organisms clouded bouillon up to and including that containing 7 per cent sodium chlorid.

TECHNICAL DESCRIPTION

***Bacterium coronafaciens* Elliott (var. *atropurpureum* n. var.)**

Variety agrees with the species in morphological and cultural characters. Differs in appearance of lesions and host range. Lesions on type host, *Bromus inermis*, have moderately developed to no halos, and oval to linear, purplish black centers 1 to 5 mm. in diameter. Artificial inoculations of *Avena sativa* produce similar lesions except that the centers are translucent not dark. In contrast, lesions on oats caused by *Bacterium coronafaciens* have small, brown, dead centers surrounded by large halos. Hosts are *Bromus inermis*, *Bromus carinatus*, numerous other species of *Bromus*, oats, and quack grass (*Agropyron repens*). In contrast the hosts of *Bacterium coronafaciens* are oats, *Bromus carinatus*, wheat, rye, and barley.

Habitat. North Dakota and Wisconsin.

ADDITIONAL EXPERIMENTS ON THE PHYSIOLOGY OF THE ORGANISM

It is thought probable that, with two bacterial organisms which are almost alike except as to infectivity, extended physiological experiments would show distinct differences, if carried far enough, especially along cultural lines. With this in view, the following investigations were conducted with *Bacterium coronafaciens* and *B. coronafaciens* var. *atropurpureum*. Although no further definite differences were discovered, certain additional data on the two organisms were obtained.

Indicator Media

Saccharine media containing mixtures of brom cresol purple and cresol red were prepared according to recommendations in the "Report of the Committee on Descriptive Chart" (2). Eight agar slants of each medium were inoculated with the brome-grass organism and eight of each with *Bacterium coronafaciens*. Both organisms changed the color to the same degree in the same length of time in each medium. Acid reaction was produced in the media containing dextrose, or saccharose as carbon compounds. Alkaline reaction was produced in those media containing lactose, maltose, mannit, or glycerine. Controls were unchanged.

Fermentation Tubes

One per cent peptone water (Dunham's solution) with 2 per cent respectively of dextrose, saccharose, maltose, lactose, mannit, and glycerine were used to make up the carbon compounds. Nitrate bouillon and milk also were used. Good growth was obtained in the open arm of all tubes by inoculation with the brome-grass organism. At the end of 30 days no gas was produced and no growth occurred in the closed arms. Table 2 shows the Fuller scale and pH readings taken during the course of the experiment.

TABLE 2

Reactions of different media before and following inoculation with Bacterium coronafaciens var. atropurpureum

Media	Reaction before inoculation	Reactions following inoculation		
		20 days	30 days	50 days
Dextrose	(Fuller's scale = + 6.5	+15.0	+18.0	-----
	(pH values = 6.5	4.9	4.4	-----
Saccharose	(Fuller's scale = + 6.0	+15.5	+16.0	+17.5
	(pH values = 6.6	5.0	4.9	4.9
Maltose	(Fuller's scale = + 7.5	+ 5.0	+ 3.5	+ 3.0
	(pH values = 6.8	8.2	8.4	8.5
Lactose	(Fuller's scale = + 4.0	+ 3.0	+ 1.0	-----
	(pH values = 6.6	8.4	8.4	-----
Mannit	(Fuller's scale = + 4.0	+10.0	+ 3.0	-----
	(pH values = 7.0	8.2	8.4	-----

TABLE 2—Continued

Media	Reaction before inoculation	Reactions following inoculation		
		20 days	30 days	50 days
Glycerine	(Fuller's scale = +10.5	+ 6.0	+ 3.5	+ 3.0
	(pH values = 6.6	6.6	7.0	8.4
Milk	(Fuller's scale = + 9.0	+ 6.5	+ 7.0	+13.0
	(pH values = 6.7	7.39	7.55	7.2
Nitrate bouillon	(Fuller's scale = +10.0	+ 5.0	+ 3.0	—
	(pH values = 7.0	8.2	8.5	—

Reduction of Nitrates

As the organisms produced ammonia, probably from peptone, when grown in bouillon, no final conclusions could be reached from the first experiments in which ordinary nitrate bouillon was used. The organisms were then grown in a synthetic medium containing small quantities of phosphate, calcium, chlorine, etc., with KNO_3 as a source of nitrogen and sucrose as a source of carbon (2). Good growth on this medium was obtained. Tests with sulphanilic reagent for nitrites and Nessler's reagent for ammonia gave negative results. These results, together with the fact that no gas is produced in fermentation tubes containing nitrate bouillon, lead to the conclusion that nitrates are not reduced.

Optimum Reaction and Toleration Limits

Beef peptone bouillon was adjusted to each of the following reactions with sodium hydroxide, and hydrochloric acid + 27 (pH = 3.8), + 17 (pH = 4.8), + 12 (pH = 5.2), + 5 (pH = 7.2), 0 (pH = 8.6), - 4 (pH = 9.0), - 10 (pH = 9.1), - 15 (pH = 9.6), - 20 (pH = 9.8), - 30 (pH = 9.9).

Optimum reaction for growth + 5 (pH = 7.2). Limits of growth were + 12 (pH = 5.2) and - 15 (pH = 9.6).

Susceptibility to CuSO_4 and HgCl_2 . The experiments were run in triplicate and the average results presented in table 3.

TABLE 3

Effect of CuSO₄ on Bacterium coronafacuum var. atropurpureum

Dilutions expressed as ratio of CuSO ₄ to H ₂ O	Number minutes treated	Number of colonies developing in petri dishes containing 10 cc. of nutrient agar inoculated with the following amounts of the various CuSO ₄ dilutions, in 10 cc. portions of which had been placed a uniform amount of inoculum		
		4 mm. loop	3 mm. loop	1 needle
0 (Control)	5	487	375	5
0 (Control)	10	503	450	8
0 (Control)	15	750	502	11
1:50,000	5	0	0	0
1:50,000	10	0	0	0
1:50,000	15	0	0	0
1:100,000	5	5	0	0
1:100,000	10	0	0	0
1:100,000	15	0	0	0
1:500,000	5	163	0	0
1:500,000	10	16	0	0
1:500,000	15	5	0	0

In a similar experiment, using mercuric chlorid in place of copper sulphate, the numbers of colonies developing in the controls were practically the same as recorded for the controls in table 3. No colonies developed in the remainder of the experiment except an average of 9 in the 1:500,000 dilution using the 4-mm. loop and allowing an exposure of 5 minutes.

Brome-grass Organism in Relation to Host Tissues.

Infection occurs through wounds and stomata. Spraying unwounded leaves of *Bromus inermis* with a water suspension of the organism results in numerous infections. A study of razor sections of young lesions obtained in this way shows that infection may be stomatal and is at first intercellular. Studies of stained sections of diseased and normal tissues which were killed in absolute alcohol and embedded in paraffin show that in old lesions the cells are misshapen, disrupted, and usually crowded full of bacteria. It is probably because these parenchymatous cells are so completely filled with bacteria at this stage that the lesions

do not present a collapsed appearance with sunken areas as is the case with the lesions of Halo-blight of oats.

Overwintering and Dissemination of the Brome-grass Organism

No special work has been done on this phase of the problem. Isolations from old material, together with field observations on the recurrence of the disease each year at the same places, lead to the belief that the organism overwinters in the lesions on the dead brome-grass. It is suspected that this disease is transmitted by the seed.

SUMMARY

1. The bacterial blight of brome-grass sometimes produces spots on the glumes and causes abundant dark purplish-brown spots on the leaves. The spots may or may not be accompanied by yellow halos. The upper nodes are occasionally killed.

2. Natural infections have been found on *Bromus inermis* and *Agropyron repens*.

3. Artificial inoculations show that the white bacterial organism found constantly associated with the disease is capable of infecting 23 species of the genus *Bromus*, oats (*Avena sativa*), and quack grass (*Agropyron repens*).

4. The organism isolated by the writers has the group number 211.-2323023. The same number is suggested as the correct number for *Bacterium coronafaciens*.

5. *Bacterium coronafaciens* in several experiments produced no infection on *Bromus inermis*. The lesions produced on oats by the two organisms are not identical.

6. On account of the differences in infectivity between *Bacterium coronafaciens* and the brome-grass organism, the latter is considered a variety of the former and given the name *Bacterium coronafaciens* var. *atropurpureum*.

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U. S. DEPARTMENT OF AGRICULTURE.

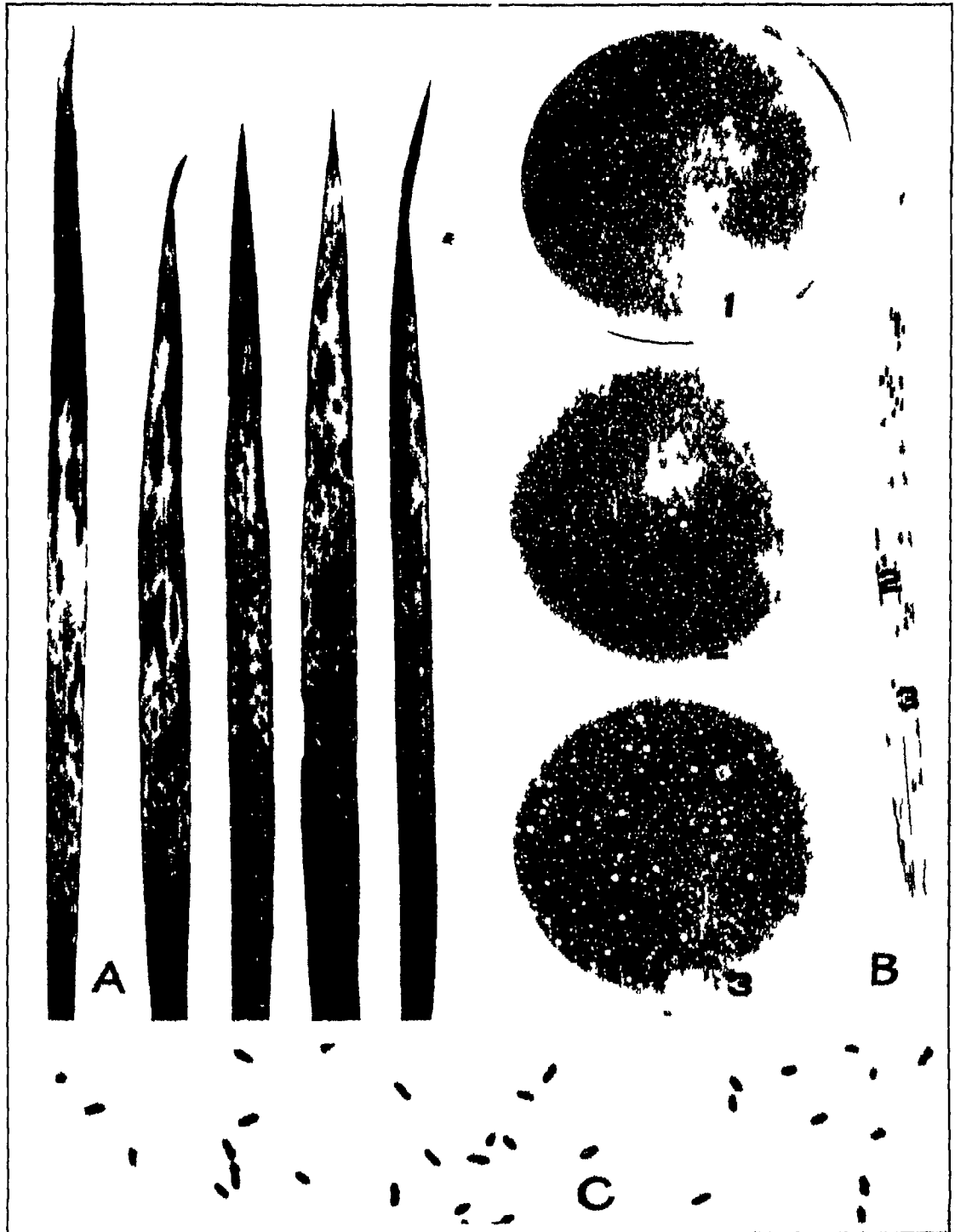
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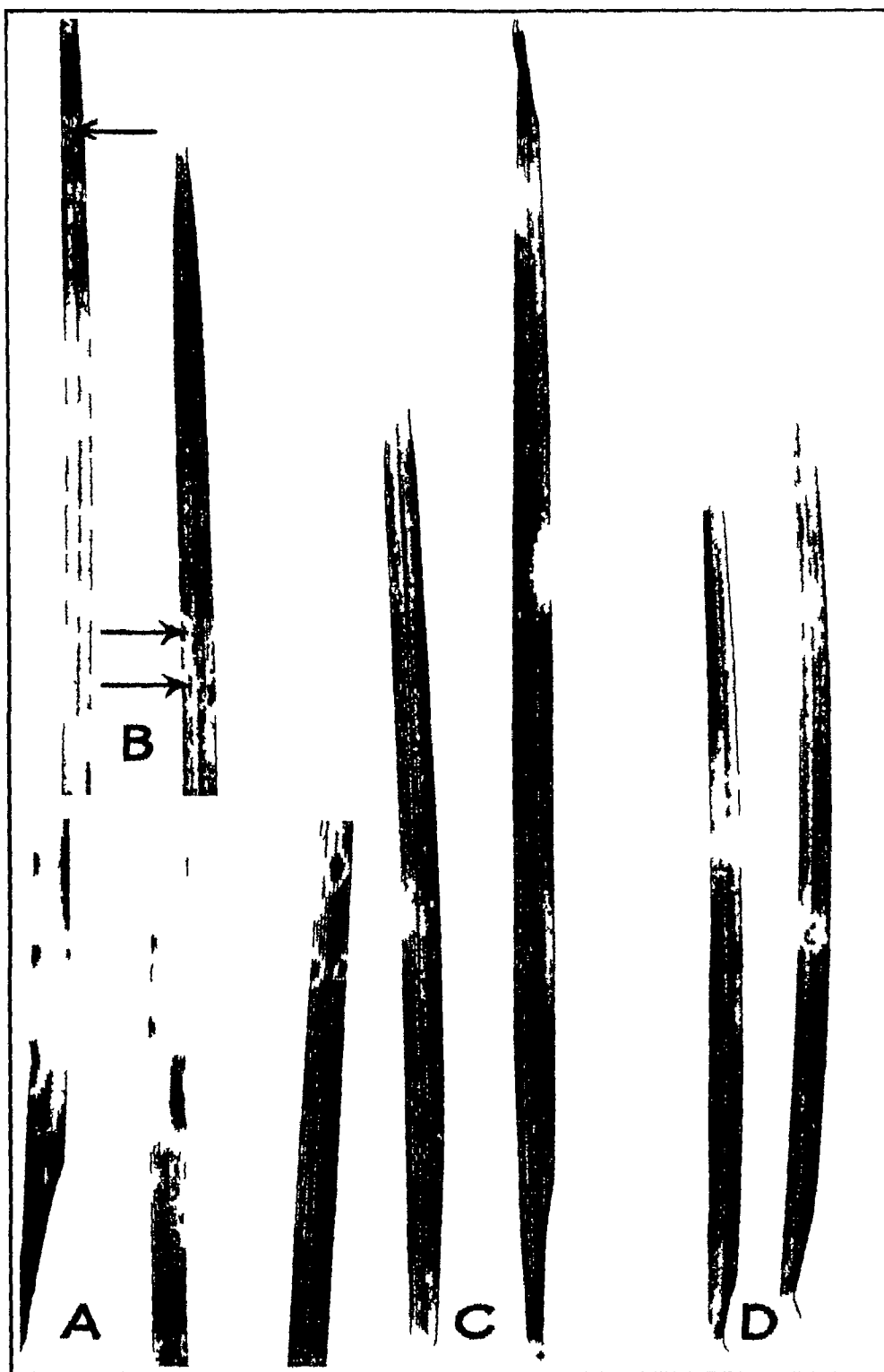
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BACTERIAL DISEASE OF BROMO-GRASS

A Typical bacterial lesions on leaves of bromo-grass (*Bromus ciliaris*) from natural infection. Marginal halos vary from moderate to none. B Colonies of the organism (*Bacterium coronafaucis* var. *atropurpureum*) in first plates of isolations from leaf lesions on bromo-grass indicated by corresponding numbers. The methods of isolation are described on page 76. C The organism (*Bacterium coronafaucis* var. *atropurpureum*) stained by Zettinow's method to show flagella.



BACTERIAL DISEASE OF BROME GRASS

Lesions produced by spraying a water suspension of *Bacterium coronafaciens* var. *atropurpureum* on unwounded leaves of A *Bromus inermis*, B *Apophyon repens*, C *Bromus secalinus* and D *Bromus hordeaceus*.

EXPERIMENTS WITH PARIS DAISY AND ROSE TO PRODUCE RESISTANCE TO CROWN GALL

NELLIE A. BROWN

WITH PLATES III AND IV AND FOUR FIGURES IN THE TEXT

Our early work with the crown-gall organism was carried on largely with the Paris daisy, and as the facilities were favorable for some studies on resistance, an experiment was started in the year 1907 with this end in view.

The plan devised was to start with daisy plants which were perfectly healthy, to inoculate them with the crown-gall organism and when the galls were full sized and the wood of the plant in condition for making cuttings, to remove slips from these galled plants. When the cuttings were full-sized plants, they were to be inoculated with the same strain of *Bacterium tumefaciens* or a strain known to be very infectious, and so on through a number of generations with the hope that resistance would eventually develop.

The work was carried through in two series of experiments, one extending from 1907 to 1912 which included 7 sets of cuttings, and 8 sets of inoculations; but of these no group photographs were made; the second experiment extending from 1912 to 1917 with 5 sets of cuttings each of which had been galled by pure culture inoculation. (Pls. III and IV.) The method used was needle prick inoculations throughout the experiment.

The details of the experiments are as follows:

FIRST SERIES OF EXPERIMENTS WITH PARIS DAISY

March 8, 1907. Twelve healthy Queen Alexandra daisy plants, propagated from cuttings, were inoculated with *Bacterium tumefaciens*. Galls formed readily and were 1.5 to 2 inches in diameter in two months. Cuttings were then made from these plants and in July when well-rooted and growing vigorously they were inoculated. Large galls formed at once and in November a set of cuttings was made from these plants. The November cuttings did not grow well during the winter but started a rapid growth in the spring and were inoculated in April, 1908. For some reason the galls were slow in forming and as the plants were in

good condition it was thought perhaps some resistance to the organism had developed. It was August before the galls were of any appreciable size; cuttings were rooted from these galled plants and were inoculated in November, 1908.

Whenever cuttings from diseased plants were inoculated other plants of the same variety (the Queen Alexandra daisy) were inoculated as controls. The controls were selected from plants which had never before been inoculated.

The plants inoculated November 18, 1908, which were expected to produce galls of the 4th series in line of successions from the original plants, failed to produce galls in the usual time. The control plants growing under similar conditions had galls in less than a month. This 4th series of plants was inoculated again, but even nine months afterwards there were no galls at the inoculated places. Suspecting immunity was becoming established, cuttings were made from these plants in March, 1909. (5th set.) In May, 1909, these new cuttings were in good condition for inoculating. The same old strain of *Bacterium tumefaciens* used before was used again, also another strain isolated from a peach gall.

In July, 1909, galls had formed on the controls whether inoculated with the old daisy strain or with the peach strain, but not on the plants in the series with either strain. This showed that the old daisy strain was still infectious and the circumstance seemed to point again to the establishment of resistance.

Next a new and very infectious peach strain which had been tested out was tried on the suspected resistant cuttings. No galls formed on them (16 in all) while control daisies of a new Queen Alexandra stock were readily infected.

On December 3, 1909, the 5th set of cuttings was tried a third time. Thirty-one plant cuttings of this series were inoculated with the daisy strain; as controls 6 young Queen Alexandra daisy plants never before inoculated were used, also 8 sugar-beet plants.

December 28, 1909. The control daisy plants had galls an inch in diameter and two months later the control sugar beets had galls 3 to 4 inches across, but there was only one daisy of the cutting series with a gall.

January 18, 1910. In the 5th series inoculations made December 3, 1909, several of the plants which showed negative results after 30 days were now producing galls. In two more months all of the 31 plants had galls of varying sizes. These plants had some resistant tendency which

was overcome as growth continued. Such resistance had never occurred before,—galls usually started on young growing plants in from one to two weeks after needle prick inoculations.

Cuttings were made in the spring of 1910, from the last (5th) set of galled plants. The 6th set in the series did not get into condition for inoculating until winter. The organism used for inoculating was an isolation from a gall on a plant of the 5th set of cuttings; its virulence had been proved on other plants (the supposition at the time of isolating was that the colonies would not be infectious but this one colony proved to be more virulent than any strain from the Paris daisy previously isolated).

On January 12, 1911, plants of the 6th set of cuttings were inoculated with this virulent strain. It was named the "Resistant Daisy" strain, to distinguish it from other daisy strains in use, as it was isolated from a gall on a plant which for a time according to appearances had acquired resistance. This strain is still in use (1921) and is still virulent, while the organisms of other earlier and later daisy isolations have weakened or lost their virulence entirely. This strain (Resistant daisy) inoculated into the 6th series of cuttings produced galls rapidly, of a large size and for the first time in the course of the experiment secondary galls were formed on stems and leaves (Fig. 1). This affected the plants badly in growth and looks. The control inoculations which were made on new plants, purchased in Boston and Springfield, had large galls also, but no secondary galls. The galls started to form more quickly on the controls than on the plants of the series test.

Cuttings were made from the plants with secondary galls, (6th set) in the summer of 1911, making the 7th set from galled plants. The growth of these cuttings was slow,—the plants seemed not to be vigorous in the fall and it was not until January 7, 1912, that they were inoculated. At this time they had started a good growth. This was also true with the 6th set of cuttings, the slips of which were slow to take root and slow in getting a good growth started. At the time the 7th series was inoculated (Jan. 7, 1912), which included 27 plants, controls were held on other new Queen Alexandra daisy plants from Boston, never before inoculated. As was true with the 6th series, galls formed more quickly on the control plants but ultimately were no larger than the galls in the series test. The control plants themselves looked much stronger however than the plants in the series test. In fact, when it was time to make new cuttings from the 7th set, the plants were in such poor condition that none could be obtained. The plants continued to look weak



Fig 1 Two Queen Alexandra Daisy plants belonging to the 6th set of cuttings in the first series test, inoculated with organism isolated from gall on previous set in series. Inoculations made January 13, 1911, with strain called Resistant Daisy. Photographed March 22 and 27, 1911, respectively.

all summer and in August all were dead but one. This plant, was set aside for later cuttings, but through a gardener's mistake it was discarded with the rest.

Discussion of results:—The death of the plants in the series test was rather a surprise, the full significance of which did not become clear until it had occurred again—namely that the constitution of the plants had become weakened through the continual production of tumors in successive generations of cuttings, so weakened that death followed.

In the six years that work had been carried on with galled daisy plants it had been found that they did not die from the presence of galls unless the galls encircled the stem. In former cases the plants were not galled in succession. The encircling stage was rather rare. The galls in the series test had not encircled the stem although the plants had died. The controls were alive and in fair condition with full sized galls on them. The conclusion therefore was that death of the series plants had resulted from a weakened condition due to a continual diseased state induced by the presence of galls on each of seven successive generations. And instead of a resistance to *Bacterium tumefaciens* being constitutionally established in the daisy plants an increased susceptibility had developed.

It remained for further tests to be made along this line to see if this susceptibility could be repeated.

SECOND SERIES OF EXPERIMENTS WITH PARIS DAISY

The second series of experiments was started in July, 1912, by making cuttings from galled control plants of the Queen Alexandra daisy, the plants never having been galled but this one time.

In January, 1913, the cuttings of this set were in good growing condition and the plants were inoculated January 7, 1913. Forty-nine plants were inoculated using the strain of *Bacterium tumefaciens* called "Resistant Daisy," which as before stated had been isolated from one of the 5th set of galled plants in the former "resistance test." In March, 1913, galls two inches in diameter had formed on the daisies. Cuttings were now made from these plants.

December 2, 1913, plants of the 2d set of cuttings (28 in number) were in good condition and were inoculated with *Bacterium tumefaciens* (3d set of inoculations with "Resistant daisy strain.")

February 3, 1914. Galls $\frac{1}{2}$ to $\frac{3}{4}$ inches in diameter were on the plants. In May of the same year all of the twenty-eight plants inoculated had produced galls. Cuttings (3d set) were made from these plants in July.

December 15, 1914. Thirty plants of the 3d set of cuttings were inoculated. Controls were held on 13 daisy plants never before inoculated.

Galls were forming well on the series plants in February, 1915. In May, 1915, photographs were made of 27 plants of this set (galled 4 times) and also of 12 of the controls (Pls. IIIA, and IIIB; IVA and IVB). The photographs show a marked contrast. The series plants were stunted and some of them dying. Three of the 30 could not be found. They had in all probability died and had been thrown out by a gardener. The control plants, bearing galls for the first time, were in good condition.

In May, 1915, after photographing, cuttings were made from the plants which had been galled the 4th time. December 20, 1915, the resulting plants (22 in number) of this 5th set of cuttings were inoculated with the Resistant daisy strain of *Bacterium tumefaciens*.

Twenty Queen Alexandra daisy plants never before inoculated were inoculated for controls. These last were grown under the same conditions as the other plants. Galls formed rapidly on all. In a week small tumors were visible. In a month (January, 1916) all the plants, series and controls, had galls three-fourths of an inch in diameter. This made the 5th set of galls in the series. In May, 1916, cuttings were made. They did not root very readily and struggled along through the summer. In the fall they were all dead but one. This one was unhealthy but it improved somewhat during the winter of 1917, and in the spring cuttings were made from it. In June, however, all these cuttings of the 6th set were dead and in the fall the parent plant died also. The cuttings never developed well and so were not inoculated. The inoculations, therefore, of this series ended with the fifth.

Discussion of results:—The results of the second experiment were like those of the first—no decided resistance to *Bacterium tumefaciens* was developed by the successive formation of galls on cultivated daisy plants, the plants used being cuttings of infected plants in successive generations. What appeared to be resistance in one stage of series I being due, it is most likely, to decreasing virulence on the part of the cultures used; the series plants requiring a more virulent strain to infect them than did the control ones. The results, however, in both series seem to indicate that the vitality of the plants was considerably reduced by repeated infection with the organism. This fact was shown by the dying of the plants after they had been galled in the line of successive cuttings five to seven times, although the cuttings made from them were taken while the plants were still in good condition.

EXPERIMENTS WITH JUICES OF GALLED PLANTS

At one time during the first series experiment, when the fifth set of cuttings after inoculation gave the appearance that resistance to the crown-gall organism was either established or becoming established, a number of the full-grown plants of this set were brought to the laboratory and treated in the following way. Stems and leaves were washed care, fully, ground, and put through the pressure filter. Varying amounts (1cc. to 5cc.) of this filtrate were added to tubes of liquid agar, the agar was then inoculated with the crown-gall organism and plates poured. The juice proved to have no permanently inhibiting effect, for typical colonies of *Bacterium tumefaciens* appeared on the plates in great numbers but when compared to control plates, made with juice from uninoculated plants, there was marked retardation in the growth of the colonies. The colonies were inoculated into healthy daisy plants, also into sugar beets. Galls formed, so the virulence of the organism was in no way reduced by the juice from the supposedly immune plants. Other experiments with the juice from infected plants were as follows:

1 to 3 cc. of the juice were added to beef agar slants, after they had been inoculated with *Bacterium tumefaciens*. Beef agar slants without the juice were also inoculated as controls. Growth was retarded in the slants with the juice, but once started the organism developed rapidly and was no less abundant than in the control plants.

To a bouillon culture of *Bacterium tumefaciens* 10 cc. of the diseased filtered juice were added also. Plates were poured from this treated culture using plain beef agar to which 3 and 5 cc. of diseased boiled juice were added to each tube. Likewise plates were poured and slant cultures were made with *Bacterium tumefaciens* using the juice of healthy daisy plants in the same way as the diseased plant juice. This juice was obtained in the same manner by passing the ground up plants through a pressure filter. Healthy daisy plants juice was also boiled without passing through the filter and then added to agar as in the test with the diseased plant juice.

Results:--The results showed that the organism grew in the media to which diseased daisy plant juice (either passed through the filter or boiled), and healthy daisy plant juice had been added respectively. No difference could be detected except in the time of the appearance of growth of the organism in the cultures. The organism in those cultures with diseased juice added was slower in making its appearance.

INOCULATIONS WITH DEAD CULTURES

An interesting experiment was one in which dead cultures of *Bacterium tumefaciens* were injected into the fibro-vascular bundles of daisy plants and the plants then inoculated in the internode above the injected area. Others on the other hand were inoculated in the spot in which dead cultures were injected. Galls formed on every stem in the former case in three weeks, but in the latter out of 11 plants inoculated only 2 produced galls and these did not appear until four weeks after inoculation.

That the culture was dead was proved by transferring some of it to bouillon and Uschinsky's solution. No development of the organism followed.

The details are as follows:

December 28, 1909. Bouillon cultures of *Bacterium tumefaciens* were heated to 60° C. for 10 minutes then injected after cooling into young Queen Alexandra daisies. About 3 cc. of the dead cultures were injected in each of 24 shoots. The needle was thrust downward in the fibro-vascular bundles. The next day another injection of a dead culture was made in the exact location as before. Immediately afterward 13 of the plants were inoculated by needle pricks in the internode just above the puncture made by injecting the dead culture while eleven of the stems were inoculated in the injection puncture.

Two plants not injected were inoculated as controls.

January 11, 1910, galls had formed on the controls. Three weeks after inoculation, galls were forming on internodes above the point of injection but none where inoculations were made in injection openings.

Forty-two days after inoculating two out of the eleven plants inoculated in the injected places had galls forming but these were very small in comparison to the control galls, or those on the plants where inoculations were made in the internodes above the injected areas. No further gall development occurred in the injected places

BREEDING EXPERIMENTS TO GET ROSE SEEDLINGS RESISTANT TO
CROWN GALL

The rose was used in this problem because of the prevalence of rose galls in greenhouses where roses are grown commercially for cut flowers and also for the sale of rooted cuttings. The disease occurs out of doors also but not so extensively as in greenhouses. Most varieties of roses are susceptible to tumor formation but the condition of growth of the

plant (whether growing rapidly or partially dormant) is an important feature in the character of the infection which follows. Because of this it is difficult to learn definitely the varieties which are least susceptible; facts which were considered necessary so that crosses could be made with these varieties. Inoculations were made on twenty or more commercial varieties in the hope of finding some variety that would be immune. Oftentimes the inoculations failed to take and the variety was considered one suitable for crossing, it being suspected that it might be an immune variety or at least less susceptible than some of the others. However, when inoculations were repeated later to verify former tests the conclusion would be entirely overthrown by the appearance of a destructive gall. It remained then to use those varieties which seemed to possess resistance and by crossing them to follow up their progeny with the hope that some of the resultant seedlings would exhibit resistant tendencies. It might happen that some of the seedlings would even entirely resist infection. The crosses were made with those roses which were in favor commercially. As indicated above there was little chance of any constant inherent resistance that could be depended upon in making these crosses.

A virulent strain of *Bacterium tumefaciens* isolated from rose galls occurring in a Massachusetts rose-house was used for inoculation throughout the experiment (Fig. 2A). When galls had developed on the stem or preferably were starting to form on the pedicel of a flower, crosses were made with the following varieties: Mrs. Charles Russell, Sunburst, Ophelia, Irish Fireflame, My Maryland, Pink Killarney, White Killarney, Prince Charles d'Arenberg, Lady Alice Stanley, Red Radiance, September Morn, Hoosier Beauty. This was done in the spring of 1916 and 1917.

February, 1917, five rose hips were ripe on galled plants. These were cut off and planted. An accident happened to two of them but of the three that remained, crosses were as follows: Mrs. Charles Russell $\times$ Sunburst; Ophelia $\times$ Irish Fireflame; My Maryland $\times$ Ophelia. Both Irish Fireflame and Mrs. Charles Russell had been slow in producing tumors after inoculation; many inoculations failing entirely to take.

The seeds germinated September and October of 1917, and throughout the summer of 1918, there were ten struggling plants. A year later only five were alive, three of which were large enough to use for cuttings. Later the other two puny plants died. Thirty-six cuttings were made from three seedlings, nineteen of them being from one which produced

a double rose red blossom (Mrs Charles Russell $\times$ Sunburst) two from a double cream pink (My Maryland $\times$ Ophelia) and fifteen from a single cream pink blossom (Ophelia $\times$ Irish Ineffable)

In April 1920 the plants were in good growing condition and were inoculated. Controls were held on stock rose bushes which had never

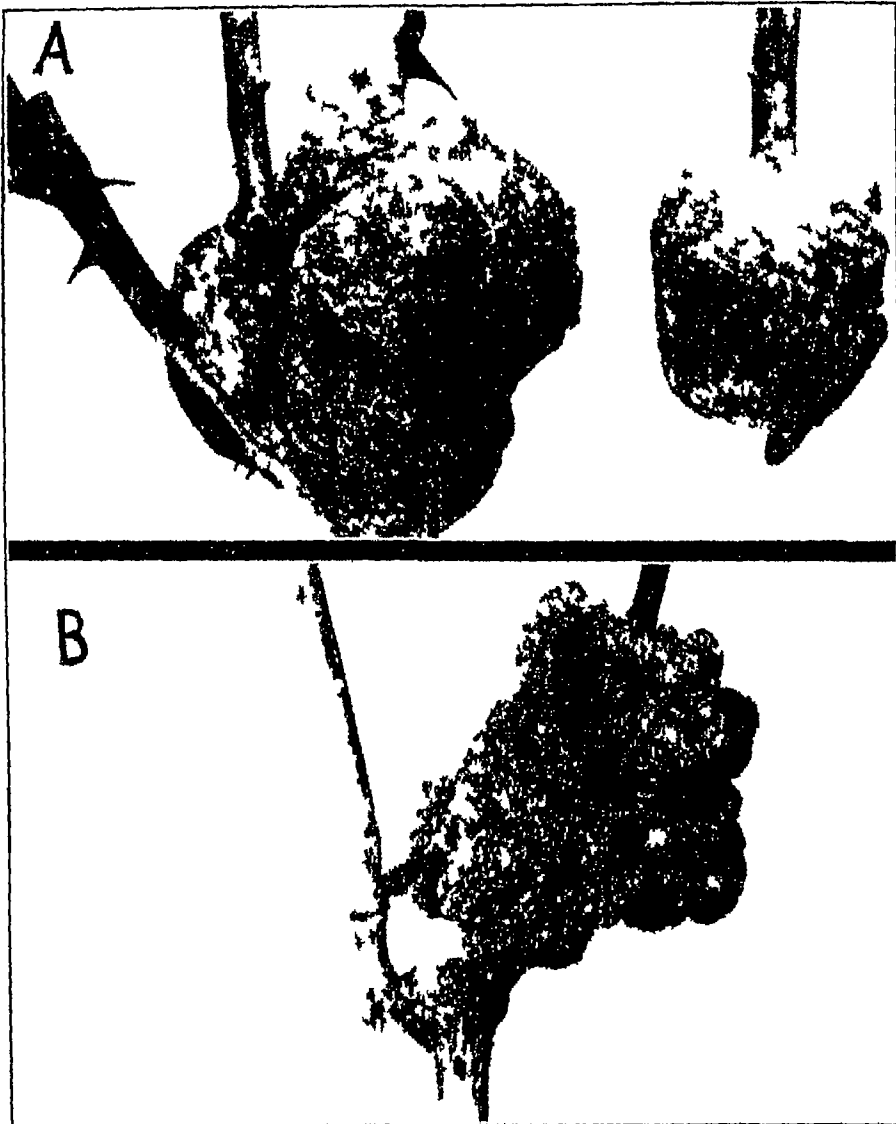


Fig. 2 A Crown galls on rose stems from Waltham Mass. April 11 1915. Natural size. Isolations made from these.

B Ophelia rosebush inoculated with rose gall organism June 2 1920 (Fig. 2a) Photographed April 6 1921. Plant had been dead two months. One of the controls in the hybrid resistance test which shows the virulence of the organism.

been inoculated. These were My Maryland Ophelia Prince Charles d'Arenberg, Sunburst, Irish Fireflame and American Beauty. Controls were also held on cauliflower plants, sugar beets and Ricinus.

In one month after inoculating galls were forming on the single cream-pink bushes but none on the double cream-pink or the rose-red bushes. In two months there were small galls 1.5 and 4 cm. in diameter on 3 inoculated plants of the double cream-pink variety and one much smaller on the rose-red. By this time a single-pink seedling gall was 2 by 3 cm. (Fig. 3a) while the one rose-red seedling gall was only 3 by 4 mm. (Fig. 3b). Galls 2.5 to 4.5 cm. in diameter had formed on the cauliflower, sugar beets and *Ricinus* (Fig. 4). On the control rose bushes they were 2 to 2.5 cm. in diameter. More control inoculations were made in May and June to learn the type of virulence of the organism during the hot months.



Fig. 3. Rose gall in hybrid immunity test. Seedling single cream-pink (*Ophelia* × *Irish Fireflame*) left; seedling rose-red (*Mrs. Charles Russell* × *Sunburst*) right. Both inoculations April 5, 1920, with same culture or rose-gall organism. Photographed June 18, 1920. *a*, gall 2 by 3 cm.; *b*, gall 3 by 4 mm. Natural size showing the temporary resistance of one variety to the crown-gall organism.

The results of the seedling inoculations at the end of July 1920, were as follows: Out of 19 rose-red bushes, (*Mrs. Charles Russell* × *Sunburst*) inoculated on many stems, 15 had no galls, even after three months; the other 4 had small galls 5 mm. to 1 cm. in diameter. Of the 15 single cream-pink variety (*Ophelia* × *Irish Fireflame*) only 8 had grown in a thrifty way, so that tender shoots could be inoculated. Six of these 8 produced galls 2 to 3 cm. in diameter, much larger than those of the rose-red variety. Of the two plants of the double cream-pink variety

(My Maryland $\times$ Ophelia) one had two large galls 2 to 4 cm.; the other plant was not inoculated. Secondary galls were later produced on the single cream-pink variety. After a year had elapsed the rose-red variety had only one sizeable gall 3 cm. in diameter while two of the single pink variety had died from the galls encircling the stem. One of the control rose bushes (Ophelia) had also died, its stem being encircled by the gall (Fig. 2B). The other controls including several Ophelia bushes had galls of various sizes but were producing flowers occasionally.

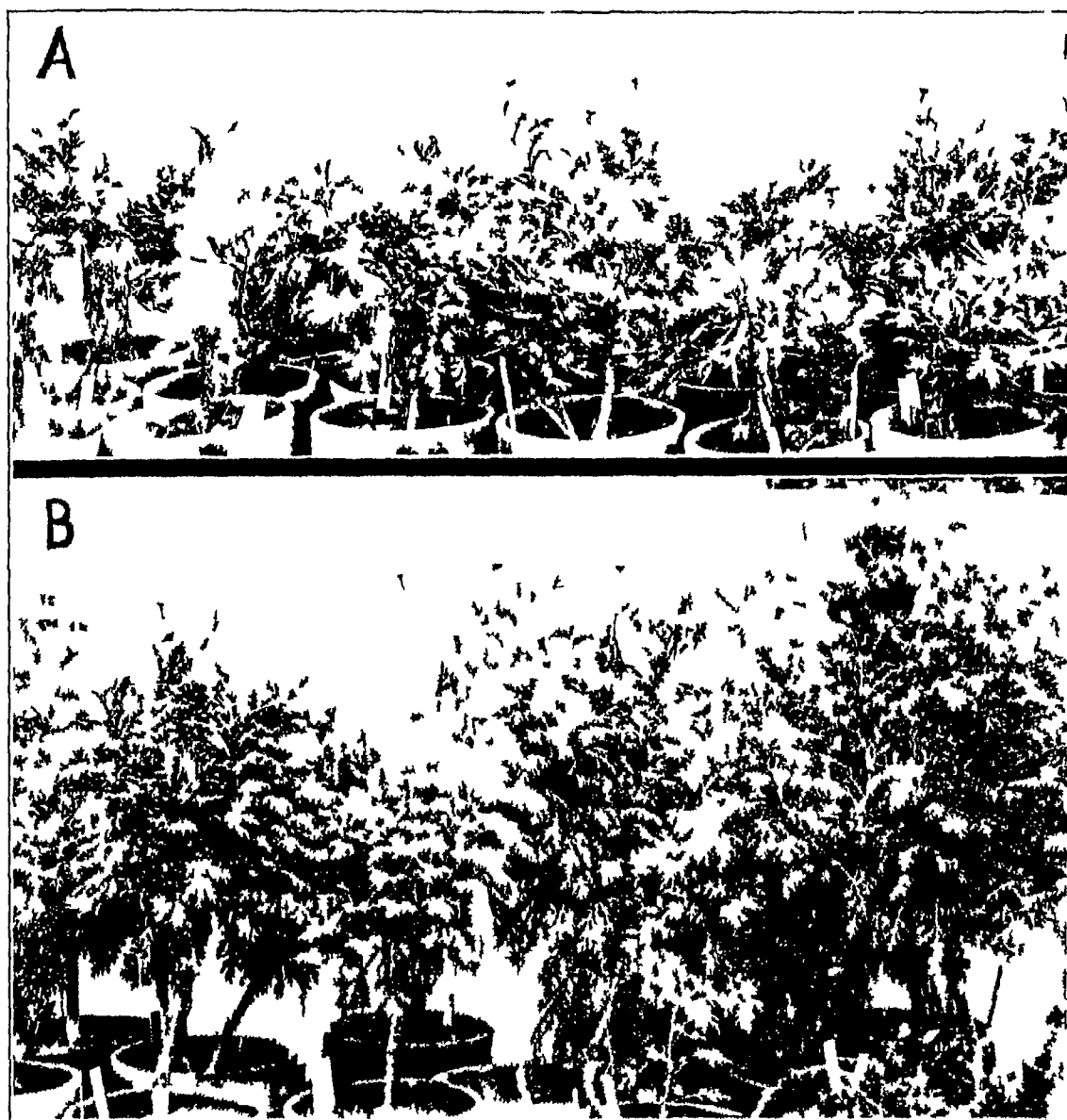
In the spring of 1921, a year after the first inoculations were made into the rose seedlings, when the bushes were sending out new growth the new spring shoots were inoculated. Special attention being given to the rose-red variety. Twenty inoculations of the rose-red variety were



Fig. 4. Rose galls on sugar beets, cauliflower and Ricinus. Plants inoculated April 5, 1920, with the Rose-gall organism, to test its virulence and as a control on the supposed resistant bushes. Photographed June 18, 1920. Reduced in size.

made; no infections followed. Galls formed readily on the single Cream-pink variety. The plants of the double cream-pink were not growing well and were not inoculated.

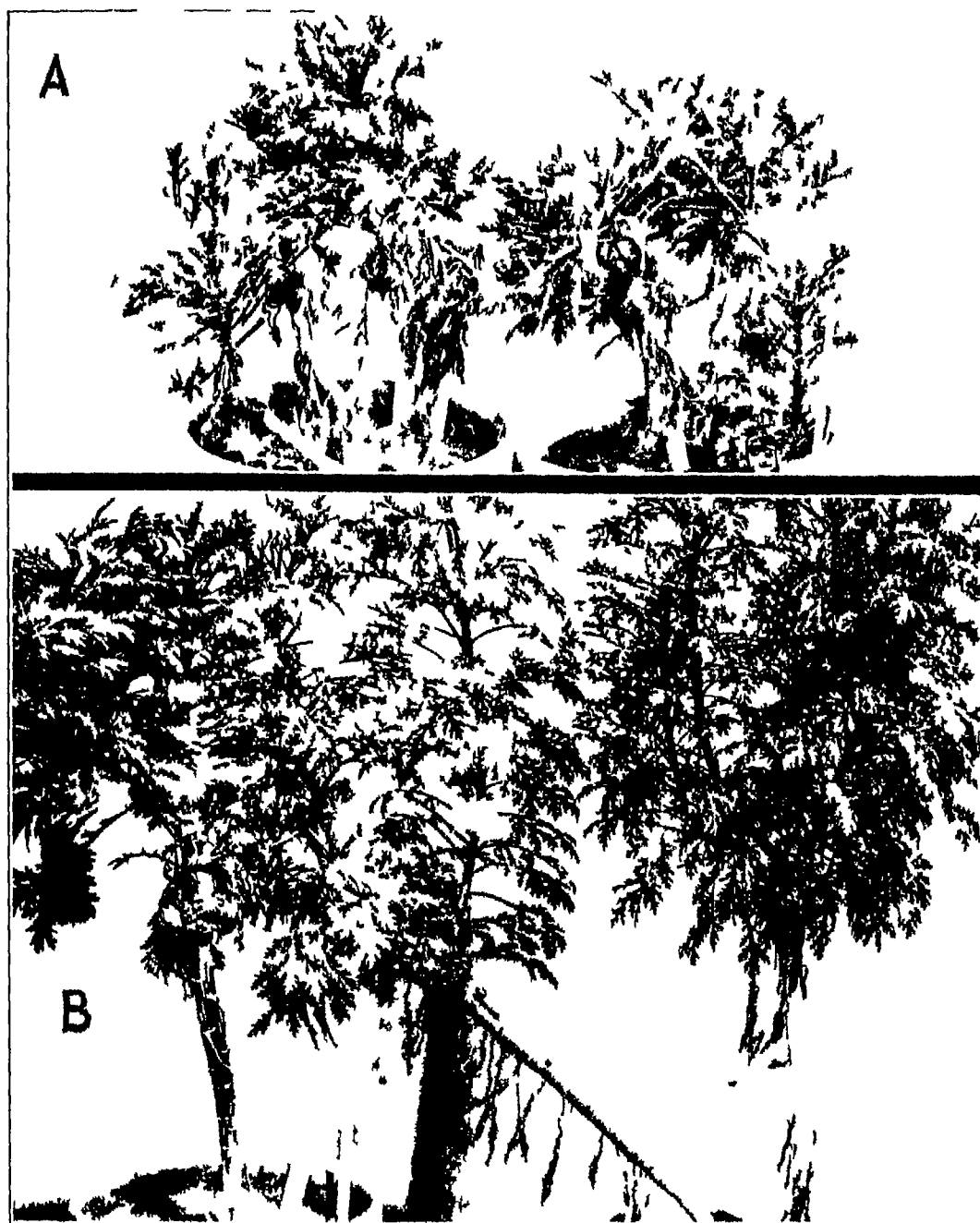
During June and July plants of the rose-red variety, (Mrs. Charles Russell $\times$ Sunburst) were inoculated again. This time galls formed in nearly 60 per cent of the inoculations showing that the resistant characters of this plant were not permanent. The galls were smaller and slower in growth than those of the two other varieties. However, they continued to increase in size during the winter of 1922 and although development was slow, in the spring they were as large as any mature galls on the two susceptible varieties.



CROWN CALL

A Dusies in second immunity and susceptibility test. Called 1 times. That is cuttings have been made from galled plants and then cuttings inoculated to the 4th series. These plants were inoculated with Dusy organism Resistant strain December 15 1911. Photographed May 3, 1915. Seventeen out of 27 shown print cut down.

B Dusy plants inoculated with Resistant Dusy Colony 2, December 15 1911. Photographed May 3 1915. These plants are controls on the immunity and susceptibility test. They had never been galled before that is they are cuttings from plants which had never had galls. Two of these were photographed out from the group. There are galls on all. The photograph shows that the plants are in good condition after 5 months when galled only once. Same magnification as A.



CROWN GALL

A. Dusies in second immunity and susceptibility test. The 4th series of cuttings called. These plants were inoculated with Resistant Dusy strain December 15, 1914. Photographed May 3, 1915. Typical plants from the lot of 27, the group photograph of which is shown in part in plate IA.

B. Dusy plants. Controls in second immunity and susceptibility test. Two cut of 12 plants inoculated with Resistant Dusy strain December 15, 1914. Photo May 3, 1915. Typical plants from the group of 12 photographed the same day (Pl. IB). These plants were from cuttings of plants which had never been galled.

SUMMARY

No permanent resistance to the crown gall organism was developed either in the Paris daisy or in rose bushes which were seedlings derived from somewhat resistant varieties. A temporary resistance or what at the time was supposed to be such was noted in the daisy cuttings in the middle of the first series, and in one plant of the three rose seedlings.

When the inoculations into Paris daisy cuttings made from galled plants were carried to the seventh generation, this resistance was lost and increased susceptibility to the disease was noted, but here a more virulent strain of the organism was used.

A second set of experiments with galled daisy cuttings seemed to indicate increased susceptibility to the disease in the fifth generation with lowered vitality of the whole plant.

Of the three rose seedlings produced from somewhat resistant plants one (*Ophelia* × *Irish Fireflame*) was very susceptible to crown gall, and one called the rose-red variety (*Mrs. Charles Russell* × *Sunburst*) appeared for a time quite resistant, that is for about a year. At the end of nearly two years this resistance had appreciably lessened although the variety never showed the same ability to form galls as did the other two seedling varieties.

THE ALTERNARIA BLIGHT OF POTATOES IN BERMUDA

H. H. WHITZEL

WITH ONE FIGURE IN THE TEXT

The following observations on an epiphytotic of early blight of potatoes in Bermuda in the early winter of 1921, may be of some interest to plant pathologists.

The fall crop consisting chiefly of Bliss Triumph and a much smaller acreage of Garnet Chili is planted in Bermuda during the latter half of September and early October. In the autumn of 1921 weather conditions were very favorable to the growth of potatoes. Warm weather and abundant rainfall combined to produce a growth of crops which by early November gave promise of an extraordinary crop as indicated by the foliage which was profuse and of a dark green color.

Early in November the weather became increasingly wet, rain falling almost daily. About the middle of the month the early blight began to appear in many of the fields all through the colony on the earlier planted potatoes. It appeared first on the lower leaves on plants here and there throughout the fields, causing them to turn yellow and die. The lesions on these first infected leaves were quite characteristic for the disease as it commonly appears in the States. The lesions were rather small, definite in outline and with the usual target board markings. Then suddenly the tops in entire fields began to die off. This often began in spots or areas which rapidly enlarged, but usually the entire field was swept as by a fire.

From a distance these blighted fields had so much the appearance of a late blight attack that I felt sure it must be that disease. The suddenness of its development, the rapidity of its devastation and the "spots" in the field all argued for the *Phytophthora* blight. Moreover, on first entering one of these fields to make a careful examination I seemed confirmed in this opinion. The lesions (Fig. 1) were large, often an inch in diameter, the margins indefinite, dark and water-soaked. There were, to be sure, here and there the smaller definite target board lesions so characteristic of the *Alternaria* blight, but they were chiefly on the lower older leaves while the large indefinite lesions were abundant all over the foliage even to the uppermost young leaves. Moreover, the green stalks showed numerous large, dark, watery lesions apparently those of the late blight.

There were however two disturbing features in these field symptoms. Such as I might not a trace was to be found of the white mildew zone so characteristic of the under surface of late blight lesions. This seemed remarkable for the ground was soaked and the foliage wet with the rains of the preceding night. A very condition requisite for an abundant production of the conidiophores of *Plasmopara infestans* seemed to obtain but none was in evidence even under a hand lens. The

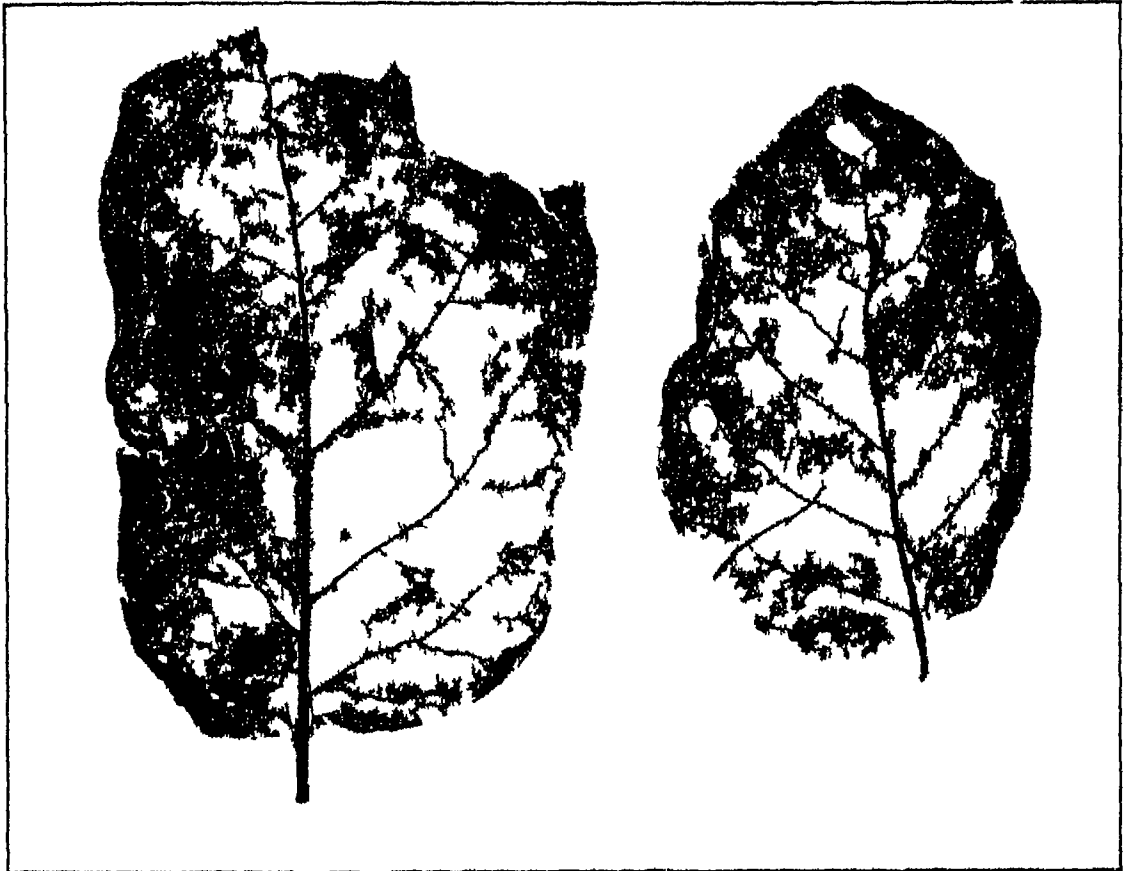


FIG. 1. *Alternaria* blight lesions on potato leaflets. Note indefinite water-soaked character of the lesions and absence of zonation. Contact photograph from dried specimens.

other puzzling thing was the slightly brownish hue and occasional traces of zonation in some of the large lesions. This together with the frequent presence of typical early blight lesions on the same leaflet caused me to hesitate over a final decision.

Mr. M. E. Luther, a recent graduate of the Wisconsin Agricultural College, had brought me to this particular field to diagnose the trouble and stood by awaiting my verdict. After audibly changing my mind two or three times, I finally said it must be the late blight though the

symptoms certainly lacked some of the usual features of that disease. I took the precaution, however, to gather a quantity of the affected "trees"¹ which I at once took to the laboratory for microscopic examination. To my astonishment the lesions all showed an abundance of the spores of *Alternaria solani* and not a trace of the conidiophores or conidia of *P. infestans*.

Repeated microscopic examinations of blighted leaves from different fields always yielded like results.

By the end of November fully one-third of all the potato fields were severely affected with the early blight and in many the tops were completely killed. The yield in blighted fields was reduced at least one-third and in some cases probably one-half. Only a small percentage of the growers spray the fall crop and those who do delay the first application until the "trees" have practically completed their growth. That is, they do not begin until the early blight is often starting or even well along. A few who this year sprayed early and thoroughly held the disease in check to a considerable degree. However, as spraying was generally delayed, only the upper leaves were protected and as not more than two or three applications at most were made, the disease developed on the lower unprotected leaves. Poor spraying due largely to antiquated nozzles and low pressure pumps together with the washing effects of frequent rains combined to offer but little check to the disease in most of the sprayed fields. The best yields from blighted fields did not exceed six barrels to one of seed planted, including all grades of tubers, while many fields gave as low as two or three to one. In all cases the percentage of seconds, third grade and cull tubers was high. One of the best potato growers who sprayed early and very thoroughly and whose field showed very little early blight harvested seven to one. A severe wind on December 5, 6, and 7 destroyed the tops in this field which accounts for the relatively low yield, but it serves as a rough standard by which to judge of the effect in neighboring fields killed by the blight shortly before the wind storm.

This attack of early blight was not especially associated with maturity of the tops, tip burn or other injuries to the foliage. The foliage when the epidemic broke out was in perfect condition and in a vigorous state of growth. The weather had been ideal for growth of the "trees." It was a clear case of a virulent attack of an independent pathogene upon a normal, vigorous host.

¹ A colloquial but universal use of the term in Bermuda. Applied to the tops of such herbaceous plants as potatoes, lilies, celery and onions.

Those features of this epidemic which strike me as extraordinary in my experience with early blight are: (a) the suddenness and rapidity of its development, (b) the completeness of its devastation, (c) the size of the leaf lesions and their similarity to those of the late blight, and (d) the development of large water soaked lesions on the stalks.

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SEED TRANSMISSION OF LETTUCE MOSAIC

ALLAN G. NEWHALL

As one of the minor diseases of field-grown head lettuce in Western New York, mosaic has been observed for several years. It is commonly present to the extent of from 3 to 6 per cent, but in dry seasons, when insects are abundant, it is much more prevalent. Such was the case in 1921. From 10 to 15 per cent of the heads of the second crop in Wayne County became affected by September. The disease was also very prevalent in other lettuce growing sections of the State. Its spread was easily correlated with a general infestation of aphids, these insects having been found by Jagger (8), in 1919 to be capable of transmitting the virus.

During the summer of 1921, repeated search was made for wild hosts which might aid in the dissemination of the virus or be responsible for carrying it through the winter and furnishing the primary inoculum in the spring. A number of attempts were made by Dr. H. W. Dye and the author to transfer mosaic from milk-weed (*Asclepias syriaca*), and from water dock (*Rumex britannica*) to lettuce by means of rubbing, transferring bits of diseased tissue, and by hypodermic injections. Toward the end of the season cages were built and aphids employed, but no success attended any of these trials.

For several reasons it was suspected that lettuce mosaic was carried over in the seed. In the first place an occasional mosaic plant was found in the flats planted with commercial Big Boston or Romaine seed in the greenhouse. In the second place the disease was observed in the field in May and June soon after the young seedlings developed their third or fourth true leaf. At this time aphids are not prevalent, nor are they commonly found even later on such small lettuce. In the third place, seed transmission was demonstrated to occur in a number of mosaic diseases of vegetables and field crops. Reddick and Stewart (11), and Nelson (10), have reported seed transmission of the common bean mosaic, while Gardner and Kendrick (6) found the soy bean mosaic was transmitted in a similar manner. McClintock (9) has reported seed transmission of lima bean mosaic, while Doolittle (4) and Doolittle and Gilbert (5) found the cucumber mosaic could live over in the seed of the wild cucumber (*Micrampelis lobata*), and possibly in rare instances in that of the cultivated form. Dickson (2) and Dickson McRoxtie (3) report positive results of "seed inheritance"¹ in the mosaic of several

¹ There is no indication that the disease appears in the offspring in a definite ratio or any implication by them that it is necessarily carried in the embryo.

legumes, notably *Trifolium pratense*, *T. hybridum*, *Melilotus alba* and *Pisum sativum*, as well as *Hippeastrum* species. Miss Westerdijk (12) has furthermore reported a possible case of seed transmission of tomato mosaic, but the work of Allard (1), Dickson (2), and of Gardner and Kendrick (6) does not support her view.

In order to test the possibility of seed transmission of lettuce mosaic, twelve heads of Big Boston showing typical symptoms of the disease were carefully selected in the field at cutting time, staked, and allowed to go to seed. Seed was harvested from each of these individuals separately. To test its viability a small amount was sown immediately in the field and, out of 37 plants which grew, two showed distinct mosaic symptoms one month later. This was not considered important at the time, since no precautions were taken against possible insect inoculation.

GREENHOUSE TESTS

The following winter (1921-1922) seed from 3 of the 12 selected plants was sown in fresh loam, not known to have previously grown any lettuce. After 28 days, mosaic symptoms were distinctly apparent on 27 plants out of the total population of 563 plants which grew. Several doubtful cases were noted but not counted. As a few aphids and other insects were found in the vicinity of the lettuce bench, another test was made. This time every precaution was used to eliminate all other possible agents of transmission. The soil was steam sterilized, the seed bed was covered with cheesecloth after sowing, April 5, and the house fumigated several times during the next three weeks. On examination 24 days later, April 29, a considerable number of typical cases of mosaic had appeared. A diligent search was made for aphids and other insects but none were found. On May 30, the plants were pulled one at a time and the following counts made.

Parent's number	Population grown	No. of Mosaic plants
L-3	410	16
L-4	40	0
L-5	169	2
L-7	170	1
L-8	200	4
L-9	101	3
L-11	175	6
L-12	200	13
Totals	1405	45%

In addition 35 doubtful cases were held for three weeks more, since under greenhouse conditions mosaic symptoms may be more or less masked and slow in developing. Six of these developed more definite symptoms.

Since making these tests, additional field observations in Western New York have been made the past season. Several fields of lettuce in Wayne County were examined carefully in different places in July 22. The plants were not over 18 days old and were just getting their second or third true leaf. One hundred plants were counted in each of a number of different places in three fields, and in every case from one to three plants showed the first symptoms of mosaic. No insects were found on any of these plants as the season was very wet and the plants very small.

CONCLUSIONS

These tests and field observations seem to prove that mosaic of lettuce is frequently transmitted through the seed.

Probably the seed is the most important source of primary inoculum in the spring.

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STUDIES ON PLANT CANCERS V.

LEAFY CROWN GALLS ON TOBACCO PLANTS RESULTING FROM BACTERIUM TUMEFACIENS INOCULATIONS¹

MICHAEL LEVINE

WITH PLATES V TO VII

One of the most interesting results of the study of crown galls has been the discovery and careful study of those leafy forms (abortive shoots, etc.) which Smith has characterized as plant embryoma(a. The tobacco is probably one of the most favorable plants for the study of these leafy crown galls.

In studying the virulence of various dilutions of *B. tumefaciens* in tobacco, I have had under observation large numbers of leafy crown galls that were associated with and formed a part of the crown galls that developed on the stems, in the leaf axils, and on the midveins of the leaves, and have compared them with those of *Bryophyllum* (Levine 3).

Levin and Levine (1 and 2) and Levine (3, 4, and 5) claimed that the leafy shoot may result as a differentiation of the crown gall tissue. Further evidence for this view has been obtained. Such galls are to be distinguished in my opinion from the leafy crown galls which develop when an axillary or dormant bud is inoculated with *B. tumefaciens*. This type of leafy crown gall is distinctly different from those that develop on stem internodes or the midveins of leaves.

METHOD OF INOCULATION

In studying the virulence of various dilutions of *B. tumefaciens*, as reported elsewhere, I have described the various dilutions and the methods employed. A brief description of the methods used will suffice here. The plants were inoculated in the stem at the internodes, in the axillary buds, and in the midvein of the leaf. This was done by inserting a trocar .5 mm. in diameter to the depth of 2 or 3 mm. removing the tissue and replacing it by a drop of suspension of *B. tumefaciens* with the

¹ From the Cancer Division, Montefiore Hospital, Dr. Isaac Levier, Chief.

aid of a syringe. The more common method of inoculation, that is, pricking the plant with a sterile needle dipped into an emulsion of *B. tumefaciens*, was also used. But with the former method the quantity of fluid is measured more definitely than has heretofore been done by myself or other investigators. In the first or trocar method, a larger quantity of fluid can be injected. In general, the sizes of the crown galls obtained were not affected by the number of bacteria.

The inoculation of the uppermost axillary buds of decapitated plants was tried. Approximately two hundred plants were decapitated and immediately afterward were inoculated with *B. tumefaciens*. This was done by inserting a trocar into the axil of the leaf followed by injecting a drop of the culture. Often a slanting trocar incision was made extending from the center of the pith of the cut end of the stem diagonally to the axil of the leaf into which the *B. tumefaciens* suspension was introduced.

It is known that when a tobacco plant is decapitated, a number of axillary buds from the uppermost to the third or fifth dormant bud below are stimulated to develop, the uppermost assuming the position of the primary stem. It has been shown that injury or impairment of function, caused by the development of a crown gall near an axillary bud, will cause the development of the bud (Levine 5) and data, still unpublished for galls, on rubber trees shows that the death of the apical portion of the stem due to a crown gall is followed by the development of a number of axillary buds below the necrotized portion of the stem.

The stimulation of the buds brought about by decapitation is in a sense analogous to the stimulation of bud development brought about by the removal of a Bryophyllum leaf. The question was raised whether the removal of the leaf in Bryophyllum would act as an added stimulant to shoot formation when the notches of the leaves were inoculated. The conclusion reached was that *B. tumefaciens* generally inhibits the development of buds and leaf notches rather than stimulates them. In tobacco my results are the same as in Bryophyllum. Smith (12) finds that, "A distinct crown gall stimulus exists for the dormant buds of Bryophyllum."

In all, over one thousand inoculations were made on about 350 plants.

OBSERVATIONS

As noted, the results obtained by inoculating at three different points were compared, first, the midvein of the leaf, which in Bryophyllum gave only crown galls without differentiation of leafy shoots; second, the stem internode; and third, the axil of the leaf or the stem node.

INOCULATION INTO THE MIDVEIN OF THE LEAF AND THE
STEM INTERNODE

For the study of leafy crown galls on the midveins of leaves, the tobacco is, in the opinion of the writer, the best material so far discovered.

The number of leafy crown galls so obtained is, however, relatively small. It approximates, however, under favorable conditions, the number of leafy shoots which appears on the stem internodes after inoculation. Of a great number of leaves inoculated in my cultures, only a small number reached maturity with their crown galls; the others failed to produce crown galls or withered before the crown gall was well developed. It must be remembered that by the time the young inoculated leaf becomes mature, its position is relatively further down on the stem, and more shaded so that in crowded cultures it may often wither and the development of the crown gall be thus interfered with. In all cases examined the growths on these midveins which later became leafy crown galls, began as crown galls of the ordinary type, the characteristic leafy shoot appearing later.

In inoculating the leaf midvein with *B. tumefaciens* by the trocar method a cylindrical opening is formed extending parallel with the axis of the midvein for a distance of 3.5 cm. With the development of crown gall, the tissue splits longitudinally. The growth of the crown gall is visible for two to three weeks before the diminutive leafy shoots are recognizable. The leaf structures first appear as small greenish white protuberances and then develop into recognizable small sessile leaves. It appears here as maintained by Levin and Levine, (2) and Levine (3, 4, and 5) that the leafy crown gall arises by a secondary process of differentiation. Just as the crown gall, in its earliest stages is made up of small cells, embryonic in appearance, and later becomes differentiated into various mature tissues, so by growth it may become differentiated into leaves and often modified stems and roots. Similar results are obtained when an inoculation is made by several pricks of the needle dipped into a culture of *B. tumefaciens*. Each incision is filled with a small crown gall of the globular type and later small leafy shoots develop from them. In all cases it was determined by careful observation that the leafy shoots appeared after the crown gall was well established. The formation of a cork-like layer and the appearance of a brown color over the surface of the crown gall indicates that a differentiate process is going on. Often, in the tobacco and especially in *Bryophyllum*, (Levine 3) it was noted that protuberances which were suspected of being capable of forming leafy shoots would spread out horizontally

and form flattened bodies not recognizable as leafy elements or roots. These would grow into large rounded masses and again give rise to protuberances which would in turn behave as described above. It appears that these protuberances can not be considered as definite embryonic leaves or roots, but are merely clusters of embryonic cells.

Smith's (10) figures (on plate 23) show similar types of development of the leafy crown gall. His figures show clearly a large globular crown gall from which the leafy structures develop. It appears that the size of these leafy shoots is determined in a great measure by the rapidity of the growth and differentiation of the crown gall. A good food supply will undoubtedly aid in the better development of these leafy structures. Most often the development of the globular crown gall is rapid and the differentiation of distorted woody elements cuts off the food supply so that the small leafy shoots dry up and die. It seems probable that the early death of these leafy structures on the crown galls is not a matter of senility, but is due to the limitation set on the available food supply by virtue of growth and differentiation of the crown gall proper.

Leafy crown galls on the internodes of the stem reach a much larger size than those on the midveins of the leaves. They are larger and the embryonic leaves which appear on them are more numerous and larger, than those on the midvein galls, but the same general sequence of events prevails. At times it appears that those on the stem have greater vitality. The leaves of these crown galls are relatively small and as compared with the normal and are always clustered. It seems that the greater the mass of tissue upon which the crown gall develops, the larger is its size and the greater is the differentiation of the crown gall tissues.

The leafy crown gall on the stem of the tobacco develops after the main mass of the crown gall tissue has been formed. In a number of instances, I have been able to obtain leafy crown galls, on the cut ends of stems of tobacco. These are similar to those shown by Smith (11) but in all cases a large mass of crown gall tissue is first formed on the cut end or within the body of the stem. This at first has somewhat the appearance of wound callus. A series of leafy crown galls obtained by inoculating with similar quantities of *B. tumefaciens* showing all stages in the development of the leafy shoot are shown in plate V, figures 1, 2, 3, 4, and 5.

Plate V, figure 1, represents a double crown gall at the internode of the stem of a tobacco plant inoculated in August 1921, and harvested in October 1921. The inoculation was made by perforating the stem by a trocar incision and *B. tumefaciens* was then introduced. Two con-

fluent crown galls developed. Each has a scaly surface and no indication of leafy shoots.

On the other hand the galls in plate V, figures 2, 3, 4, and 5 show a number of leaves varying in size and degree of development. Yet these plants were grown practically under the same conditions and inoculated similarly, and have produced equally large globular crown galls. In the first (Pl. V, fig. 2) the shoot on the crown gall consists of a leaf like protuberance "a". In plate V, figure 3, several leaf-like buds may be recognized while in 4 there has formed a structure which has a leaf and stem-like appearance. In plate V, figure 5, the galls produced by a perforating incision also have developed quite recognizable leafy bodies. It is of interest to note that while the globular crown galls are rather uniform in size, the size and number of the leaf-like elements vary.

A considerable number of plants showed swellings, comparable to the smooth galls described earlier (Levine 5 and 6). These galls are smooth in outline and covered by apparently normal epidermis. Sections through these crown galls show a hyperplasia of a particular tissue such as pith, wood or cortex, and the growth of the epidermis appears to keep pace with the growth of the internal tissues. The growth of the epidermis which covers the internal hyperplasia is not determinate, for it eventually cracks, necrotizes, and its scales form a part of the gall. An injury caused by a needle prick at the time of inoculation inhibits the growth of the epidermis at that point. The crown gall appears to grow through the opening and what appears to be callus tissue forms around the wound. Numerous cases of this phenomenon appeared in internodal inoculations of the tobacco.

The leafy crown galls so far described as noted form a group of crown galls which result from the further differentiation of the so-called globular type of crown gall.

INOCULATION OF STEMS AT THE AXILLARY BUDS, PLANTS NOT DECAPITATED

The effect of the inoculation of the axillary bud of the tobacco with *B. tumefaciens* was studied extensively. The growth that develops here is of two types. First, the globular crown galls formed are associated with a comparatively elongated branch-like growth which is undoubtedly the result of an abnormal development of the axillary bud. I have not made cultures to recover the organism from these shoots, but the presence or absence of the bacterium in the tissue would not necessarily indicate whether the parasite is the cause of its development. Never-

theless, this type of crown gall, when it occurs, is a distinct entity and may be distinguished from the leafy growths which appear on the surface of the globular crown gall by its position on the stem, namely, in the axil of the leaf. This axillary leafy crown gall as we shall refer to it may develop synchronously or appear somewhat later than the globular hyperplasia.

Plate VII, figure 10, and plate VI, figures 11, 12, 13, and 14 show a more or less graded series of axillary growth. In all cases, the axillary leafy shoot is associated with the crown gall, often growing through it; more commonly the leafy growths appear on a dwarfed atypical stem as shown in plate VII, figure 10, and plate VI, figures 11, 12, and 13. It not infrequently occurs that the axillary shoot appears to be quite normal. This occurs when the inoculation is made at the side of the axillary bud (Pl. VI, fig. 14). The development of this shoot is not to be construed as directly caused by the organism, but rather indicates a vascular disturbance affecting the distribution of food, thus calling forth this growth, possibly in the same way that decapitation would do.

It is of interest to note that in plate VII, figure 10, the crown gall appears to be an abortive stem from which aberrant leaves appear, having a more or less cyclic arrangement. In plate VI, figure 12 the branch marked "a" can be recognized as the axillary shoot. It is stunted, yet the arrangement of the leaves can be definitely recognized. Plate VI, figure 11 represents a similar condition; "b" is the axillary shoot the base of which is surrounded by globular crown gall which differentiated into leafy appendages. Plate VI, figure 13 shows still further the size to which the axillary leafy crown gall may develop.

The most striking characteristic of the growths of this type which arise from the axillary bud when inoculated with *B. tumefaciens*, is that the structures so formed are never as well developed as an axillary shoot arising from a decapitated stem, but are dwarfed, fasciated and abnormal in appearance. The leaves are often pale green and in a measure resemble the leaves of the other type of leafy crown gall. These leafy structures vary in size and development and form a series of growths varying from an typical branch to one almost normal in appearance (Pl. VI, fig. 14). These leafy crown galls may be regarded as the typical axillary leafy crown gall.

That *B. tumefaciens* does not always cause the development of this axillary type of leafy crown gall may be seen from the great number of inoculated axillary buds which do not produce these leafy shoots. This constitutes the second type of cases. It appears in the case that the

inoculation of *B. tumefaciens* into the axillary bud may inhibit partially or completely the development of the dormant bud. In plate V, figures 6, 7, 8, and 9, we have a few of the large number of cases of this sort obtained by inoculating the axillary buds. Large globular crown galls have been produced, while the dormant buds have failed to develop. Such types of development predominated my cultures of *Bryophyllum* (Levine 3). It is perhaps possible that under these conditions the entire bud rudiment is deformed and is either given over to crown gall cell formation or is engulfed by the rapidly growing cells that form the crown gall. In all these cases, the leafy crown gall of the differentiated type, that is, the form with small leaves coming from the surface of the crown gall may also be present.

The development of the axillary leafy crown gall may be due at first to a stimulation brought about by increasing the food supply. The young crown gall cells may be responsible for this by stimulating the transportation of food supply, thus causing the development of the axillary bud. The development is then retarded and the shoot is dwarfed or stunted when compared with the shoots from axillary buds stimulated by decapitation. A careful study of a large number of these growths confirms the opinion that the crown gall organism while it may indirectly stimulate the development of the dormant bud, prevents its full normal development.

INOCULATION OF THE STEM AT THE AXILLARY BUD OF DECAPITATED PLANTS

My further test of the effect that the crown gall organism has on an axillary bud when stimulus such as decapitation is added, gives results similar to those I have already described for *Bryophyllum* leaf notches. In the latter case, the leaf notches were stimulated to shoot production by removing the leaves from the plant and placing them in moist soil. The control leaves uninoculated produced at the leaf notches large normal shoots, while those leaves inoculated at their notches with *B. tumefaciens* produced relatively large crown galls and diminutive or no shoots at all. Smith's (12) figures also show this to be the case for leaves not detached from the plant. Whether there is a summation of the stimuli to growth resulting from the two sources, viz. decapitation and inoculation, is not altogether clear from either my results or those of Smith. It is not easy to make exact comparison of the number and exact character of the growths initiated under the two sets of conditions. It is of course perfectly clear that the final influence of the organism is to dwarf and deform the growths which do start in the inoculated regions

but it is quite possible that the stimuli to cell growth and division from the two sources are summated though the further effect of the organism when present is to prevent the harmonious biogenetic interaction of the cells produced which would lead to the formation of a normal leafy shoot. That the inoculated buds produce a smaller total growth in the end may be merely due to this resulting malformation which prevents normal assimilation and transportation of food stuffs.

In the case of the tobacco, decapitation leads to the development of a number of axillary buds so that at the end of the summer season a large decapitated plant may produce from two to five large branches arising from the upper axillary buds. The inoculated axillary regions produce crown galls with malformed branch-like growths. The elements of the axillary shoots are recognizable but the characteristic normal development does not appear. The shoots are generally stunted; the internodes are short, and the leaves appear to be aberrant, small and of a pale green color. The mechanical injury concomitant with the inoculation may also have a part in disturbing the bud rudiment. A number of small branches may appear with the crown gall. These leafy shoots appear to develop synchronously with the crown gall and form a component part of it.

Plate VII, figure 15 represents a case in which the axillary shoot developed but slightly after inoculation. Here the crown gall "b" seems to partially surround the cut end of the primary shoot "a," which has been cut close to the axil of the leaf; "c" represents the development of the axillary bud. Compare these aberrant branches with the diameter of the branch arising from the axillary bud "d" uninoculated. For purposes of photography the shoot "d" was removed. The diameters of the two are sufficient to show that the uninoculated axillary bud develops into a normal branch, which when highest on the stem, as is well known, assumes the position of the primary shoot, while the inoculated axillary bud produced here a large crown gall with a small stunted shoot ("c").

In another plant shown in Plate VII, figure 16, the largest in my cultures, similar results were observed. The axillary leafy crown gall produced several shoots all of which were smaller than the five axillary leafy crown gall type, dwarfed and pale green. The leaves were attenuated, also pale green in color and dried at the tips.

The crown gall in this case was very extensive, the plant bearing it was the largest that appeared in these cultures. This is in line with the evidence (Levine 6) that the better the health of the host, the larger the size of the crown gall. The gall is covered by abortive leafy outgrowths.

This combination of the leafy crown galls and the axillary leafy crown gall, an aberrant development of the axillary bud often appeared.

It is as noted very obvious that while inoculation with *B. tumefaciens*, like decapitation, acts as a stimulant to cell growth and division, the parasite when present regularly leads to rather degenerate further growth and poor development as compared to the growth of an axillary bud due to stimulation by decapitation without inoculation.

SUMMARY

I. (1) Two types of leafy crown galls are distinguished in the tobacco plant when inoculated with *B. tumefaciens*.

(2) The first type has already been described and figured as globular in form and covered with small leaf-like structures which result from the differentiation of the crown gall tissue. Such galls appear on the inoculated midveins of the leaves and on the stem internodes of tobacco.

(3) The second type of leafy crown gall has been referred to in this paper as the axillary leafy crown gall. It appears often when the axil of the leaf is inoculated with *B. tumefaciens*. The axillary leafy crown gall consists of rather elongated stem as compared to the leafy crown galls of the first type, and results from the aberrant development of the axillary bud.

(4) It is not certain that the growth of this leafy shoot is due directly to the presence of *B. tumefaciens*. The stimulus to growth may be the result of a mechanical modification of the food supply incident to the development of the crown gall tissue.

(5) The axillary leafy crown gall is small and atypical as compared to the shoot from an axillary bud stimulated to growth by decapitation.

(6) An added stimulus such as decapitation does not increase the size of the axillary leafy crown gall when the axillary bud is inoculated with *B. tumefaciens*.

DESCRIPTION OF PLATES

Plate V. Figures 1, 2, 3, 4, and 5 show a series of crown galls produced by the inoculation of the internodes of the stems of tobacco with *Bacterium tumefaciens*. The crown galls show a graded series of leafy developments on the globular mass of the neoplastic tissue. In figure 1 the leafy shoots are barely visible. In figures 2, 3, and 4 the size of the leafy protuberances are larger. In figure 5 the apical portion of the crown gall on opposite sides of the stem are profusely covered with well differentiated leafy shoots.

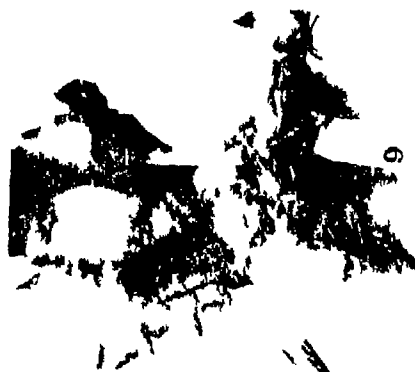
Plate V. Figures 6, 7, 8, and 9, show a series of crown galls produced by inoculating the axil of the leaves with *B. tumefaciens*, the development of the axillary bud in each case is inhibited.

Plate VI. Figures 11, 12, 13, 14 and figure 10 plate VII show crown galls in the axils of leaves with various stages in the development of the axillary bud. The familiar globular portion of the crown gall is covered with leafy shoots.

Plate VII. Figures 15 and 16 show the extremes of the effect of inoculation of the upper axillary bud of a decapitated tobacco plant with *B. tumefaciens*. In figure 15 the axillary bud development represents a large globular crown gall through which a small aberrant leaf-like stem developed; the leafy crown galls of the differentiated variety may also be seen at the base of the gall. Figure 16 represents a longitudinal section of a tobacco plant through the crown gall. The cut end of the stem is covered with differentiated crown gall tissue consisting of numerous leafy shoots. The axillary bud appears to have grown through the mass of crown gall tissue into two aberrant stem like structures.

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THE SPHAERULINA LEAF SPOT OF CLOVER

H. F. HOPKINS¹

WITH PLATES VIII AND IX AND THREE FIGURES IN THE TEXT

This leaf spot was first observed by the writer in the spring of 1920 on the campus of the University of Missouri. The resemblance between it and a leaf spot of bur clover caused by a species of *Pleosphaerulina* previously studied by the writer in Alabama is very striking and suggested the possibility that this also might be caused by a species of this same genus. A search of the dead clover leaves near infected plants showed that many of them bore perithecia similar to those of *Pleosphaerulina*. The ascospores, however, showed no longitudinal walls as in the latter. The species is, therefore, a *Sphaerulina* and has been determined by the writer as *Sphaerulina trifolii*. E. Rostr. From a close study of the literature it appears that the disease has not been previously reported in America and for this reason it will perhaps be of some value to give an account of observations and experiments made with this leaf-spot since it was first found.

Evidence that the disease was present in this locality at least as early as 1902 was obtained on examining a specimen of *Trifolium repens* in the phanerogamic herbarium of the University of Missouri. It was noted that some of the lower leaves of this specimen bear the typical minute young lesions of the *Sphaerulina* leaf-spot. A microscopical examination of these small spots showed them to be the same as those described below and also to have usually near their center the empty walls of the ascospores from which the lesions had developed. Thus far the disease has been found on white clover (*Trifolium repens* L.), alsike clover (*T. hybridum* L.), and on red clover (*T. pratense* L.). Data from inoculation experiments indicate that mammoth clover (*T. pratense perenne*) is also susceptible, white sweet clover (*Melilotus alba* Lam.) and yellow sweet clover (*Melilotus officinale* Willd.), bur clover (*Medicago maculata* Willd.), and alfalfa (*Medicago sativa* L.) are probably immune. The disease has been found most commonly by the writer on white clover in the vicinity of Columbia, Missouri. Other collections have been made at Rocheport, Clayton, St. Charles, and Blue Springs, Missouri; Ithaca, N. Y., and Godfrey, Ill. The writer has found it in nearly every growth of white clover examined. The loss caused by it is probably not great, although a considerable amount of defoliation may take place under favorable conditions.

¹Published with the approval of the Director of the Missouri Experiment Station.

SYMPTOMS

The disease is exhibited at first as minute black lesions on the leaf blades, petioles, and stipules. These lesions are very small and easily distinguished from spots caused by *Phyllachora trifolii* (Pers.) Fekl. At this stage the leaf has the general appearance of being peppered. The spots are slightly depressed especially on the petioles and on the under sides of the leaves. Both the upper and lower sides of the leaf usually bear lesions. Leaves have been found frequently, however, with lesions only part way up on the petiole and others with only the petiole and under side of the leaflets infected. The presence of numerous lesions of this sort often causes the leaflets to turn yellow and fall. When the leaflets turn yellow the dark lesions stand out in strong contrast. Even if there are but a few young lesions on a leaf they are more readily distinguished by holding it between the eye and a light. Such young lesions are shown in plate VIII, figure 1, photographed by transmitted light. Later these small spots enlarge and have a light brown to gray center surrounded by a dark reddish brown margin as shown in plate 1, figure 2. The perithecia may sometimes be seen with the unaided eye in these older lesions. By holding the leaf up to the light and examining it with a hand lens the perithecia stand out clearly as small bright bodies in which one may often distinguish the ostiola. Under conditions of continued high humidity the whole of the infected leaflet may become water-soaked in appearance and develop large numbers of perithecia throughout the tissue. In the case of red clover, perithecia develop readily on the stipule lesions.

Besides the parts mentioned above, lesions occur also on the peduncle, calyx, and corolla. Infection on the calyx seems significant in that it points to a means by which the seed may become infected and thus transmit the pathogene. Young lesions on the petioles and peduncles are usually elongated vertically but are otherwise similar to those on the leaf blades. Infections have not been found by the writer on the stems of either white or red clover although numerous lesions have been observed on peduncles and petioles in close proximity thereto, so that although it seems that the stems must have been exposed to ascospore discharge they did not become infected. Abundant infection has been found on the stems of alsike clover. In one instance minute lesions on red clover stems which did not appear to be typical of the *Sphaerulina* disease were observed. Isolations from these lesions, moreover, gave rise in all cases to pure cultures of another organism.

ETIOLOGY

Nomenclature. The fungus was determined by the writer to be a species of *Sphaerulina* and was then compared with herbarium specimens of *S. trifolii* E. Rostr. on *Trifolium repens* (No. 790 Sydow Mycotheca germanica). The two are identical both as to the lesions produced on the clover leaves and as to the morphology of the organism. The limits of ascospore length as given by Rostrup¹ are somewhat smaller than those determined by the writer for his own material. The former places the limits as 32 to 33 by 12 to 15 μ while measurements of ten ascospores of material collected here vary from 30.4 μ to 39.5 μ with an average of 35.1 μ . At the same time the spore width was found to vary from 12.2 μ to 15.1 μ with an average of 13.2 μ . As it is seen this agrees very well with Rostrup's limits. As shown in figure 1, B, the mature asci are considerably longer than wide. Rostrup gave their measurements as 50 μ in diameter and did not give values for the length and width. The measurements of asci gave a variation in length from 64 μ to 71 μ with an average of 72 μ and in width from 33 μ to 50 μ with an average of 43 μ . Measurements of the herbarium specimen mentioned above were made and are listed below along with Rostrup's measurements and measurements made from the writer's own specimens.

	Present collections	No. 790 Mycotheca Germanica	Rostrup's measurements
Perithecia	80-125 μ	98-102 μ	Not given
Asci	61-71x33-50 μ	17-59x30-41 μ	50 μ diameter
Ascospores	30.4-39.5x12.2-15.1 μ	25.2-29.4x11.2-12.6 μ	32-33x12.15 μ

It seems evident therefore that the ascomycete under consideration is the same that Rostrup observed on white clover in Denmark and is *Sphaerulina trifolii*.

Morphology. The mycelium of this species as it occurs in infected tissue is usually rather large in diameter, is densely granular and frequently shows swellings. On potato agar plates it is somewhat the same but perhaps more uniform. The perithecia are outlined in figure 1, B. They are flattened slightly on the vertical axis and have relatively large ostiola. The perithecial walls are somewhat smoky in appearance but are very thin so that when the contents are mature the asci and ascospores may be seen within them. As mentioned above perithecia

¹Rostrup, E. Mykologiske Meddeleser (VIII). Sprodtte lugttagelser fra 1897-1898. Bot. Tidsskr. 22: 254-270. Illus. 1899. Bibliographical footnotes.

appear as small bright bodies when the older lesions are viewed by transmitted light with a hand lens. They are not beaked. The ascus when young is pear-shaped as shown in figure 1, B, and at the base has a much thickened wall composed of a homogeneous hyaline substance. This wall gradually becomes thinner as it approaches the larger end of the ascus in which the young ascospores are borne. Later when the spores are mature this thickened part of the wall is reduced and appears as in figure 1, C.

The ascospores are almost invariably four celled with three cross walls although a few exceptions (e. g. two and three celled spores) have been found by the writer. Some of the spores are symmetrical but a large number show a bulging out of one of the central cells. The mature spores are filled with granular protoplasm which often contains a number of large oil drops (Figs. 1, C and 2).

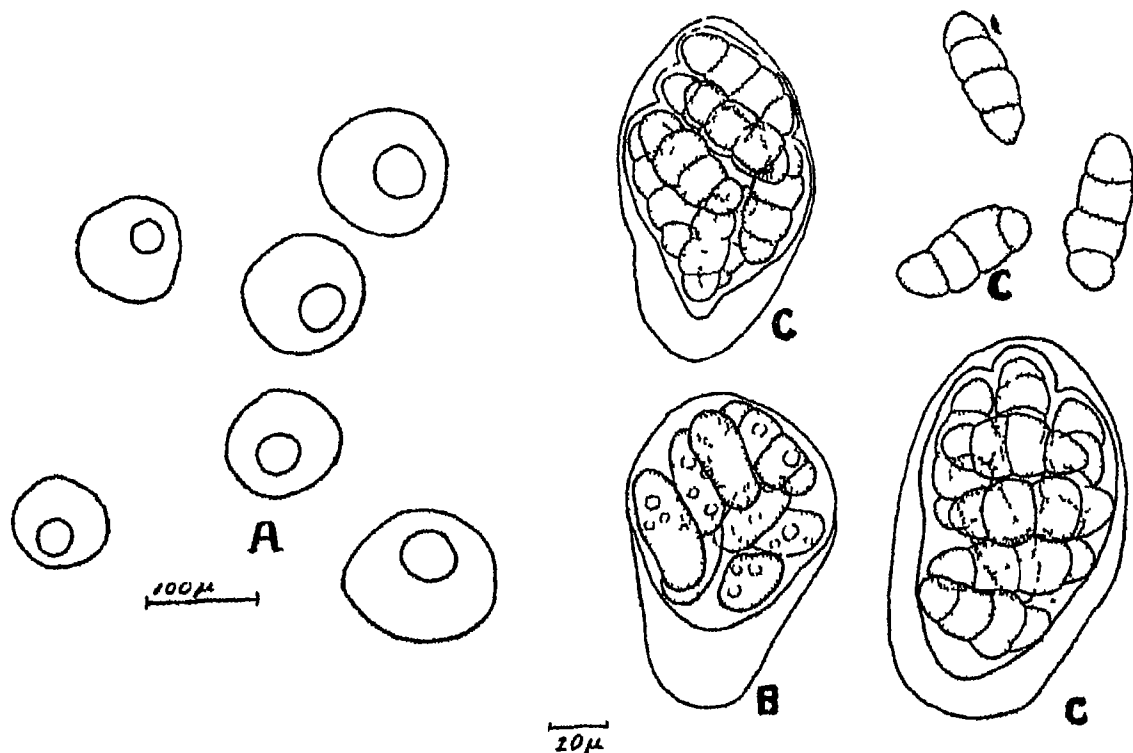


FIG. 1. *Sphaerulina triticii*: A. Perithecia from a white clover leaf. B. An immature ascus from white clover. C. Mature asci and ascospores from white clover. Camera lucida drawings.

Isolations. A large number of isolations were made from leaf blades, petioles, stems, calyces, and peduncles which showed the small lesions, and in every case the same characteristic growth was obtained on potato agar as shown in plate IX figures 1 and 2. This growth was also identical with colonies from the tissue of larger lesions and from single ascospores.

In two instances cultures of *Colletotrichum trifolii* Bain were obtained with *Sphaerulina*. Thus far no mature perithecia have been obtained in pure culture. The fungus makes a good, but slow growth on potato agar. At first the colony consists of white mycelium only, but after a few days spherical white bodies which soon turn shiny black appear at the center and finally large numbers of them are formed over the surface of the colony up to within a few millimeters of the advancing margin. At this time the fungus forms a tough membrane on the surface of the agar

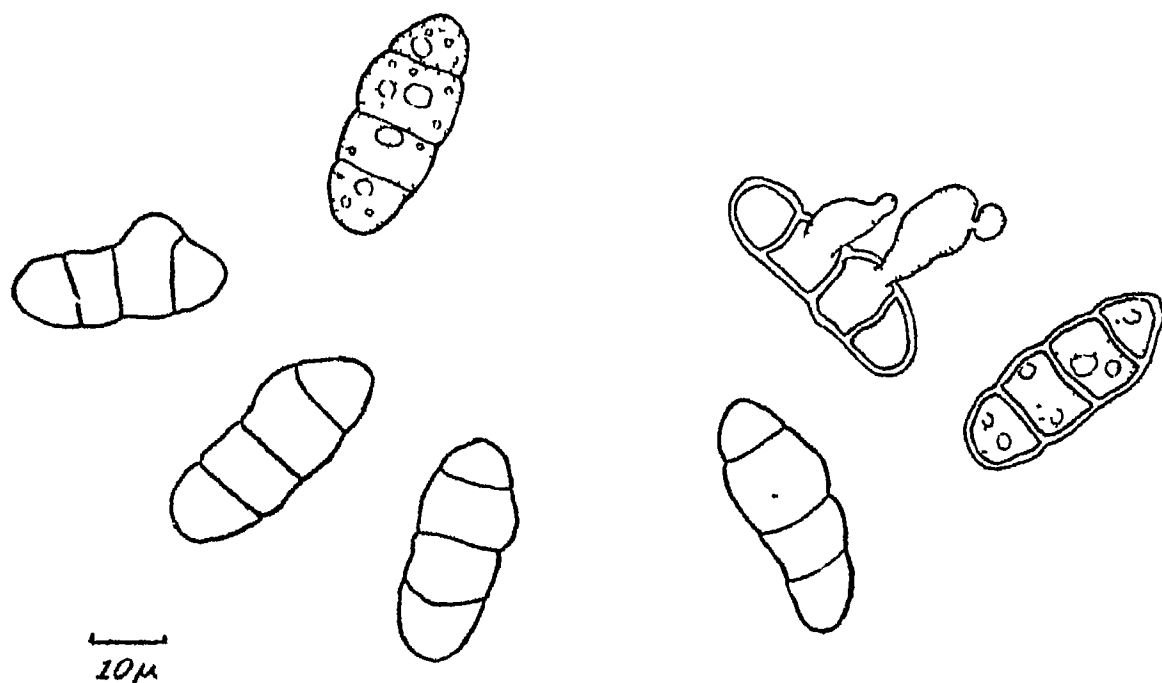


FIG. 2. *Sphaerulina trifolii*: Ascospores from the surface of an infected leaf. Camera lucida drawing.

and there is but little aerial mycelium present. The general appearance of the colony is black with a white border. A large number of microscopic examinations of these black structures failed to show any with asci, ascospores, or pycnospores. The wall is membranaceous and is made up of cells similar to the walls of the perithecia found in nature but with no ostiolum. The contents are granular and frequently contain what appear to be oil globules. In cultures on sterilized clover leaves in large tubes and preparation dishes the same black bodies were formed abundantly in the leaf tissue. In this instance as before, none of them contained mature asci. However, in some, a number of ovate hyaline structures which may have been immature asci were observed.

Cultures on potato agar when actively growing have a pleasant fruit-like odor not present in cultures of *Pleosphaerulina briosiana* to which they are otherwise quite similar. In several isolation experiments in which the growth was at first not typical the presence of the organism was indicated by this odor. Later the cultures developed normally. A strong acid reaction does not prevent growth and this fact is of value in isolating when bacteria are present. In the following experiment actively growing mycelium from a pure culture of the organism was planted on plates containing 1, 2, and 3 drops of lactic acid respectively, per 20 cc. of potato dextrose agar.

TABLE 1

Growth of Sphaerulina trifolii on acidified potato dextrose agar.

Drops of 50 per cent lactic acid	pH (approximate)	Growth in diam. mm.				
		30 hrs.	2 days	3 days	4 days	6 days
1	4.5	13.0	15.5	17	19 0	22
2	4.0	11.0	13.5	15	16.5	19
3	3.8	7.5	11.0	11	14.0	10

Even with three drops of the acid there is fairly good growth after 30 hours although the growth is only about one half that in the least acid culture. It is evident from the table that after a time the relative difference between the growth of the cultures gradually disappears. This is probably due to a change in reaction in the more acid culture plates which makes them more favorable for growth.

Pathogenicity. Difficulties were experienced in obtaining infection with mycelium from pure cultures of *Sphaerulina trifolii* because of the drying out of the inoculum before the pathogene had become established. However, in one experiment with a single ascospore culture (No. 32) and a culture isolated from infected tissue (No. 30) strong infection was obtained on mammoth clover, medium infection on white clover, and slight infection on red clover. Reisolations from the infected leaves were successful.

As the lesions produced in the above experiment were not typical an attempt was made to obtain more natural infection. Two pots of red clover plants free from leaf spots were used. Over one of the pots was placed a bell glass in the top of which were attached white clover leaves which bore large numbers of perithecia. As a control the other pot of

plants was covered with a bell glass in which no perithecia were placed. Both bell glasses were removed at the end of about 76 hours. No difference was noted at the time of uncovering, but two days later the plants exposed to the perithecia showed abundant minute lesions identical with those found in the field. After two days more, a careful inspection was made of each leaf. Of 70 leaves in the pot exposed to ascospore discharge, 52 or 74.3 per cent were infected, while none of the 60 leaves in the control plants were infected. Successful isolations of *Sphaerulina* from the spots produced in this manner were obtained. A microscopical examination of the lesions showed at their centers the presence of ascospores usually devoid of contents as is the case in field infection.

The experiment was repeated but this time 8 different species of legumes were used. They represented three genera: *Trifolium*, *Medicago*, and *Melilotus*. Plants in one pot of each were inoculated and those in one pot used as a control. The bell glasses were placed over the plants on a cold cloudy afternoon. No lesions were noticed on any of the plants by the next day, but the morning following (41 hours after exposing to the perithecia) definite and characteristic lesions were found on the leaves of all inoculated plants while none of the control plants showed any lesions. In table 2 the species are arranged in order of the relative amount of infection, beginning with mammoth clover which was most severely affected and ending with bur clover which was least infected. The numbers in the third column indicate approximately the relative amount of infection on the leaves on the basis of 10, which represents the most severely infected.

The bell glasses were removed at the end of 48 hours and the plants were placed under a cheese cloth shade in the greenhouse to protect them from the heat of the sun. The lesions on the species of *Trifolium* continued to develop in size while those on the alfalfa, bur, and sweet clovers even after 8 days did not appear larger than they were when first observed.

From the above experiment and also from the first inoculation experiment it may be concluded that mammoth clover is the most susceptible, while red and white are about equally so under conditions of our experiments, and alsike less susceptible. The writer has not had the opportunity to study mammoth clover in the field. However, under field conditions white clover is much more severely attacked than red clover. The species of *Medicago* and *Melilotus* are probably not naturally host plants for this organism, as the spots did not develop further than the pin point stage as they did in the case of mammoth, red, white and alsike

clovers. While a pure culture was not used in the last two experiments the writer considers that the pathogenicity of the organism is demonstrated.

Seasonal history. In the spring of 1922 as soon as the white clover began to grow it was inspected almost daily for the *Sphaerulina* leaf spot. No infection was found up to March 13. Previously there had been little precipitation but from March 13 to 14 there fell about four inches of rain and on the 17 one center of infection was found, and the next day others. As a check in the diagnosis frequent isolations and

TABLE 2

Results obtained on inoculating various species of Trifolium, Medicago, and Melilotus with Sphaerulina trifolii.

Host	Relative infection	Remarks
Mammoth clover (<i>Trifolium pratense</i> L.)	10	Lesions very abundant and prominent
White clover (<i>Trifolium repens</i> L.)	7	Lesions abundant and strong.
Red clover (<i>Trifolium pratense</i> L.)	7	A large number of lesions much more prominent than in the case of bur clover.
Alsike clover (<i>Trifolium hybridum</i> .)	5	A few scattered lesions.
Alfalfa (<i>Medicago sativa</i> L.)	3	Spots few but prominent.
White sweet clover (<i>Melilotus alba</i> Desr.)	3	Spots few but prominent.
Yellow sweet clover (<i>Melilotus officinalis</i> L.) Lam.	3	Few scattering lesions but more prominent than those on bur clover.
Bur clover (<i>Medicago maculata</i> Willd.)	2	A few minute lesions.

microscopic examinations were made. At this time a search was made for the source of these primary infections. A number of old dead leaves were found at the base of some of the infected clover plants. These leaves were covered with perithecia containing asci and mature ascospores. As they seemed to be in active condition there is no doubt that they were responsible for the first infections of the year. It seems plausible that they remained dormant until the heavy spring rains caused the asci to shoot their spores and initiate infections on healthy leaves. As mentioned under symptoms these minute spots gradually enlarged and turned light brown at their center where perithecia are produced. These perithecia then furnish more inoculum which causes infections throughout the season. Up to the present time no conidial

stage has been found to be connected with the species. Although several of the Fungi Imperfecti found on clover leaves have been cultured, the cultures do not at all resemble those of *Sphaerulina trifolii*. The writer has not attempted to determine to what height the ascospores are shot from the perithecia, but the following experiments show that they are shot upward at least one half centimeter. A leaflet containing perithecia was moistened with sterile distilled water and attached to the under side of the cover of a petri dish which had been previously poured with

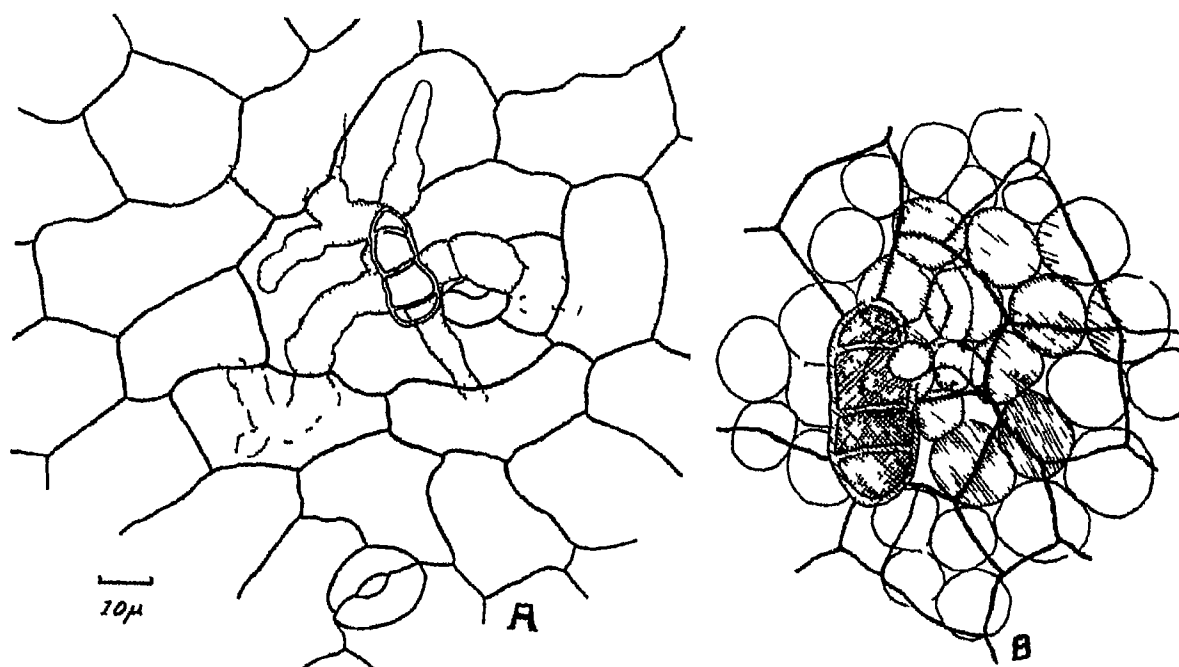


FIG. 3. *Sphaerulina trifolii*. A. Penetration of epidermis of white clover leaf. Camera lucida drawing. B. Details of early stage of infection on leaf of white clover. The ascospore and palisade cells in the immediate vicinity have turned brown.

potato agar. The plate was inverted and left over night and by the next morning (about nineteen hours) germinating ascospores were found on the surface of the agar plate directly above the leaf. The inoculation experiment discussed above shows that the perithecia readily expel their spores when plenty of moisture is present.

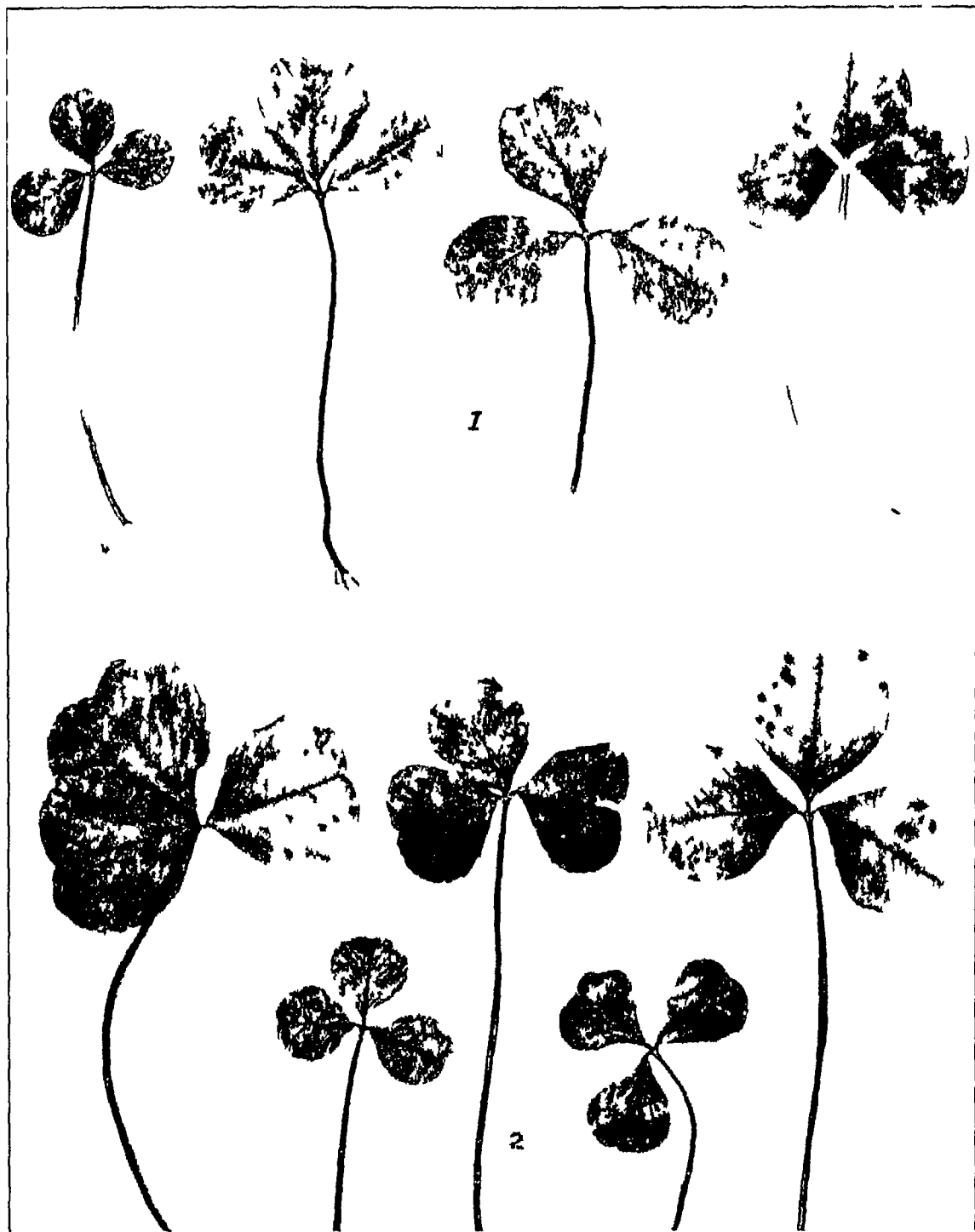
The fact that lesions have been found on the calyx and corolla indicates that seed infection may take place and this would account for the general distribution of the pathogene. Of a large number of commercial samples of white clover seed examined, mycelium was found in practically every instance in some of the seed coats. No successful isolation cultures were obtained from these seed to establish the identity of the

fungus although very short periods of disinfection were used. It was finally concluded that the samples were old and the mycelium was dead. Seed will be collected from well infected clover plants and an attempt made to settle this question.

Pathological relations. Soon after the ascospores come into contact with the leaf surface they germinate and send germ tubes directly through the epidermis. The germ tubes may grow for some distance from the spore on the surface before penetrating the epidermis, as in figure 3, A, or they may penetrate directly as in figure 3, B. No definite appressoria have been made out although the germ tubes are frequently swollen above the point where they enter the leaf. The empty walls of the ascospore persist for some time after infection has taken place perhaps because of its thickness. In about thirty-six hours after inoculation the lesions are visible to the eye because of the dark brown color which developed about the infected region. The spore itself takes on a brown color and also the palisade cells near the penetrating germ tubes. This is illustrated in figure 3, B. Finally, the color becomes so deep as to obscure the details of the infected spot. As the spot enlarges the center becomes thin and brown due to the death and drying out of the cells. The margin of the lesion still retains the deep brown color which gives the characteristic appearance as shown in plate VIII, figure 2.

The writer is indebted to Miss Marjorie F. Warner, Librarian of the Bureau of Plant Industry, for the exact citation of the article containing Rostrup's description of *Sphaerulina trifolii*.

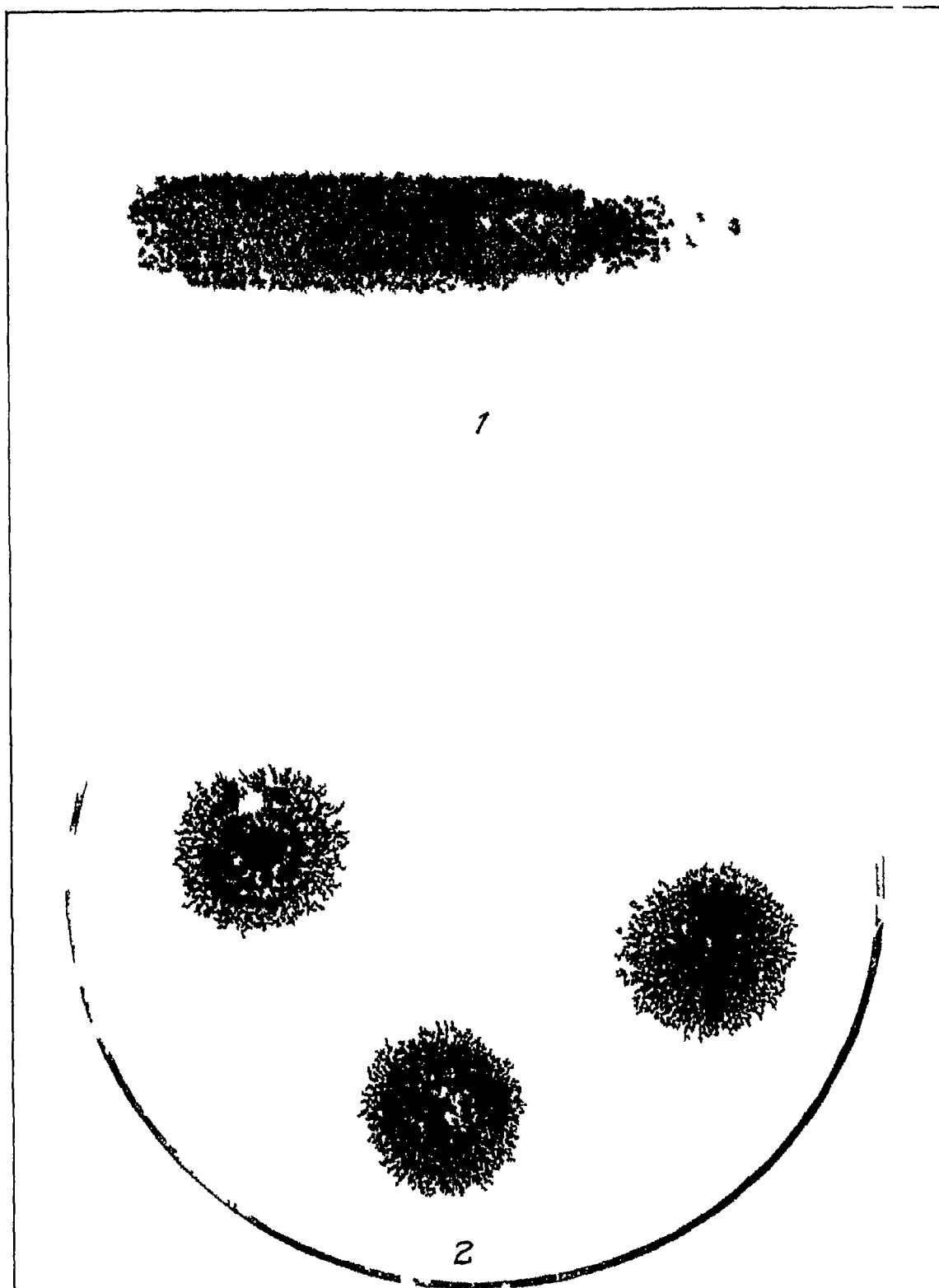
DEPARTMENT OF BOTANY,
UNIVERSITY OF MISSOURI.



SPHACULINA LEAF SPOT OR CROVLE

FIG 1 *Sphaculina* leaf spot on leaflets and leaf petioles of *Trifolium repens* Early stage

FIG 2 *Sphaculina* leaf spot on *Trifolium repens* Advanced stage Note the color about the margins of the lesions



SCLEROTINIA LEAF SPOTS OF CLOVER

FIG 1. Infected leaflets of *Sylvestris* foliar

FIG 2. An isolation plate culture of *Sclerotinia trifolii*

STUDIES ON UROCYSTIS TRITICI KOERN., THE ORGANISM CAUSING FLAG SMUT OF WHEAT

R. J. NOBLE¹

WITH PLATE X AND TWO FIGURES IN THE TEXT

Studies on the physiological relationships of *Urocystis tritici* Koern. have always been considerably hampered by the fact that the conditions necessary for spore germination were not known.

This apparently also is true for a number of other smuts. On account of scanty germination of the fungous spores, the study of the organism has frequently been limited to inoculation experiments with infested soil. The present investigations were directed toward a determination of the conditions which would result in more vigorous germination of the flag smut spores.

GERMINATION STUDIES

For several years McAlpine (3) made observations on the germination of spores of *U. tritici*. He used tap water in his tests, and records germination which varied from a "small proportion" in material one month old, to 40 per cent in material which apparently was four or five months old.

Early studies by the writer indicated that the spore germination was very capricious. Material from Australia and the United States was used in a number of preliminary tests, but, under a series of varied conditions in trials extending over periods which ranged from several days to several weeks, only a few spores germinated.

MATERIAL AND METHODS

The spores used in the following series of tests were obtained from the wheat varieties "Bobs" and "Federation," grown in the United States Department of Agriculture greenhouse, Arlington, Virginia, and collected in March 1921. When the results recorded below were obtained, this material was at least six months old; and the spores still germinated normally when they were fourteen months old.

¹ Ben Fuller Research Scholar of University of Sydney, Australia, and Graduate Student of the University of Minnesota.

The writer wishes to acknowledge his indebtedness to Professor E. C. Stakman of the Department of Plant Pathology, University of Minnesota, for advice and helpful criticism, and also to the United States Department of Agriculture for material and for granting the necessary facilities for the investigations.

It was found that most uniform results could be obtained by the use of Syracuse dishes. The spores were sown on the surface of from 2 to 3 cc. of liquid contained in the dish. Diseased leaf tissue was broken into fragments about 5 mm. in length, and the spores were shaken out through the exposed ends of the unopened sori. There was practically no contamination by other fungi. This method resulted in saving of time and made it possible to use material from the same source in a series of tests.

NATURE OF SUBSTRATE

Various organic and inorganic substrata were used. "Nutrient solutions" of inorganic salts and various sugars, carrot tissue infusions, wheat plant tissue infusions, beerwort, soil water extracts, manure extract solution, and other media were used in a number of different concentrations and under different conditions, without materially increasing the amount of germination.

A number of organic acids were used in an attempt to find some specific stimulus for germination, but without success.

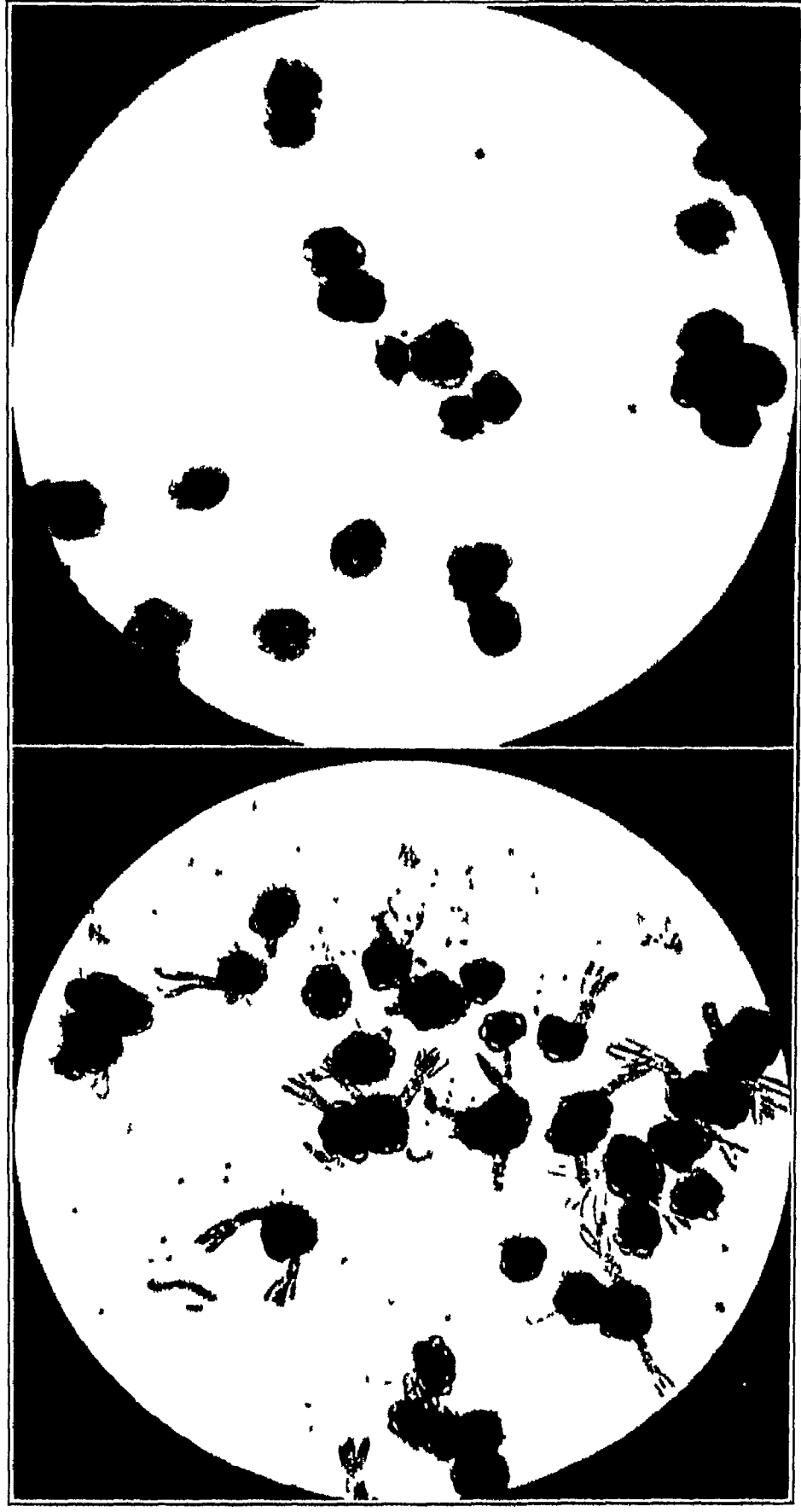
The spores did not germinate readily in any of the sodium phosphate solutions of different hydrogen-ion concentrations. Various soaps of saturated and unsaturated fatty acids were used to change the surface tension of the medium, but none of the changes appeared in themselves to be of great significance.

The spores occasionally germinated in soil water extracts and in plant tissue infusions, but even then only to a slightly greater extent than they did in distilled water. In distilled water at 22° C., a few spores occasionally germinated after 4 days, but sometimes no germination was observed until after 16 days, and frequently all the spores failed to germinate.

Subsequent tests have indicated that the maturation of the spore was not a limiting factor in these cases, for spores which were from 1 to 24 months old germinated under suitable environmental conditions.

No method of spore treatment appeared to stimulate germination consistently, until it was found that spores which had been presoaked in water for several days would germinate profusely after the addition of small quantities of wheat seedlings tissue. (A few razor sections about 20 μ . in thickness.) (Pl. X, fig. 1 and 2.)

It will be noted from table 1 that the stimulatory action of the plant material is much more potent when it is added to spores which have been presoaked for 3 or more days than when it is added simultaneously



UROCYSTIS TRITICI

FIG. 1. Germination of spores of *Urocystis tritici*. Twelve hours after addition of plant tissue to spores presoaked in distilled water. Germination at 25° C. $\times 190$.

FIG. 2. Germination of spores of *Urocystis tritici*. Spores presoaked in distilled water as in figure 1. No plant tissue added. $\times 190$.

with them. In a number of tests when dry spores were sown on wheat plant infusions, they failed to germinate, whereas presoaked spores germinated readily in the same media. The spores remained in a receptive condition for at least 28 days after sowing in distilled water, although in this case the addition of tissue resulted in only 2 per cent germination.

TABLE I

The effect on germination of adding wheat tissues to distilled water in which spores of Urocystis tritici had been presoaked for different periods of time.

Dish No.	Date on which spores were sown	Date on which tissue was added	Percentage of germination						
			Feb. 27	Feb. 28	Mar. 1	Mar. 2	Mar. 5	Mar. 7	Mar. 8
80	Feb. 24	Control	0	0	0	0	0	trace	
81	"	"	0	0	0	0	0	0	trace
82	"	Feb. 24	0	trace					
83	"	"	0	0	1				
84	"	Feb. 25	trace	2	2				
86	"	Feb. 27	0	30					
87	"	"	0	20					
88	"	Feb. 28	0	0	90				
89	"	"	0	0	85				
85	"	Mar. 1	0	0	0	65			
91	"	Mar. 4	0	0	0	0	70		

All portions of the young wheat seedlings appeared to be equally effective. The addition of uninjured wheat seedlings to dishes of distilled water containing presoaked spores also stimulated germination almost to the same extent as did the addition of small portions of plant tissue.

The action was not specific for wheat plant tissue, since the tissues of rye, barley, oats, flax and various grasses also stimulated germination of presoaked spores, although wheat plant tissue was the most effective.

Tissues derived from seedling wheat plants of the einkorn, emmer, and spelt groups, were all equally effective. No difference was observed between the action of tissue derived from wheat varieties susceptible to flag smut and from those which are resistant.

It was found that autoclaved infusions and also the distillates obtained from water extractions of wheat seedling tissue also effectively stimulated the germination of presoaked spores, provided that satisfactory dilutions were used. Dry spores sown in or on such media frequently failed to germinate.¹

The general conditions necessary for germination of the spores thus having been established, further studies were then made on the physiological relationships and the morphological characters of the organism.

RELATION TO TEMPERATURE

Fawcett (2) has pointed out that the cardinal temperatures of any organism may vary considerably, according to the conditions under which the determinations are made.

In studies on the temperature relationships of *U. tritici* it was found that the nature of the treatment to which the spores had been subjected had a considerable influence on their subsequent behavior at germination. Strong germination was always obtained 12 to 18 hours after the addition of wheat plant tissue to spores which had been presoaked for approximately 3 days at from 22° to 24° C. (Pl. X, fig. 1). It was found, however, that spores which had been presoaked at lower temperatures and subsequently incubated at 27.5° C. rapidly lost their viability (Tab. 2). The moisture-temperature relationship thus indicated is possibly a factor in causing the reduction of infection, frequently noted when wheat is sown in infested soil some time after the incidence of rain.

Further studies on the temperature relationship were made at a fixed temperature range. The spores were sown on the surface of distilled water in Syracuse dishes and incubated for 3 days at temperatures which ranged from 7° C to 27° C. On the addition of portions of wheat plant tissue, it was found that germination commenced first at 18° C, then it occurred successively at 22° C, 12° C and 7° C, none being noted at 27° C.

¹ Since the preparation of this manuscript, Brown (Studies in the physiology of parasitism IX. The effect on the germination of fungal spores of volatile substances arising from plant tissues. Ann. Bot. XXXVI: 294. 1922) has recorded the stimulatory effect of distillates derived from water infusions of the leaves of *Ruta* and *Pelargonium* on the germination of spores of *Bolrytis cinerea*.

It was found that there was very little difference in the percentage of germination after continuous incubation within a temperature range of from 19° C to 24° C, but that the growth of germ tubes was a little more rapid at the higher temperatures.

In a number of tests involving the use of spores which had been presoaked at 20° C, it was found that a few spores presoaked for from 6 to 8 days germinated even at 29° C; and, in one case, a few abnormal promycelia were formed at 32° C, but none of the spores germinated at these temperatures after they had been presoaked for only 3 or 4 days.

The effect on germination of presoaked spores of Urocystis tritici of incubation for varying periods at 27.5° C. prior to the addition of wheat plant tissue.¹

Dish No.	No. of hours at 27.5° C.	Percentage of germination at 20° C.
76 C	3	80
76 D	6	72
76 E	17	21
76 F	21	25
26	21	20
27	30	trace
28	30	0
76 G	41	0
29	45	0
66	control ²	90
30	"	80

With reference to low temperatures, it was found that germination occurred at 5° C, although this test involved the use of ungerminated spores which had been presoaked for 6 days at 20° C (Tab. 3). In one

¹ Spores presoaked for 5 days at 20° C.

² Controls at 20° C. throughout. Tissue added to all dishes simultaneously. Dishes removed to 20° C. after incubation at 27.5° C.

test it was found that the sporidia grew slowly even at 0° C. A dish of spores in the earliest stages of germination was placed in a water tight container and then totally immersed in a mixture of ice and water. The sporidia had germinated after immersion for 24 hours.

In a temperature range test with presoaked spores, it was found that the maximum growth of germ tubes took place at a temperature of 24° C, although there was very little difference in the percentage of germina-

TABLE 3

*The effect of temperature on the germination of spores of Urocystis tritici*¹

Dish No.	Temp	Percentage of Germination				
		Apr. 10	Apr. 11	Apr. 12	Apr. 13	
98	5°C	0	0	0	80	Promycelia in early stages of growth
99	5°	0	0	0	75	"
100	13°	0	0	98		"
1	13°	0	0	95		"
2	18°	0	90			Average length of promycelia and sporidia 30μ
3	18°	0	92			"
4	20°	0	90			" 10 μ
5	20°	0	95			"
6	21°	0	85			" 105 μ
7	24°	0	91			"
8	27°	0	82			" 45 μ
9	27°	0	90			"
10	32°	0	trace			Few abnormal germ tubes
11	32°	0	0			

¹Spores sown on distilled water April 4 at 20° C and transferred to wheat plant decoction and to incubators April 10.

tion throughout the range, 18 to 27° C. Under these conditions the maximum temperature apparently is just below 32° C, and the minimum temperature is between 0 and 5° C. (Tab. 3).

It will be observed that spores germinated vigorously at 27° C; but, from previous tests, it is known that constant incubation at temperatures above 25° C is extremely unfavorable to spore germination. From the practical standpoint, however, it is interesting to note that germination may occur within a comparatively wide range of temperatures, but that the previous history of the spores immediately prior to germination has a considerable influence on their subsequent behavior.

It thus appears that spores which are incubated at fixed temperatures have lower optimum and maximum temperatures for germination than those which are noted for spores which have been presoaked at low temperatures before exposure to a range of temperatures.

Under field conditions the spores are subject to a constantly changing environment, and it is apparent that their behavior must vary considerably from that which might be assumed from laboratory tests, in which the spores are only exposed at fixed temperatures for extended periods.

In the absence of moisture the spores will withstand much higher temperatures than those recorded above for spores in the presence of moisture, although the extreme limits in the former case have not been determined. In the presence of moisture, however, it is noted that those temperatures which permit a period of presoaking at comparatively low temperatures considerably extend the temperature range within which germination may occur.

RELATION TO OXYGEN SUPPLY

In preliminary tests it was found that the spores of *U. tritici* rarely germinated when totally immersed in the medium in which they were sown. This fact is generally attributed to the lack of oxygen, and has been frequently noted by investigators for other types of spores. The results of Melhus and Durrell (4) with urediniospores of *Puccinia coronata* Cda. are quite characteristic of the general experience. Duggar (1) has also referred to the phenomenon in connection with the germination of the spores of certain of the Basidiomycetes.

In tests with spores of *U. tritici* it was found that those which were totally submerged in distilled water would germinate almost as readily, and to practically the same extent, as those which were sown on the surface, on receiving a suitable stimulus after a presoak period.

There was a distinct tendency for the promycelia of the totally submerged spores to become abnormally elongated before producing sporidia. The average length of the promycelium produced by submerged spores was approximately 70 microns, while the average length of the promycelia developed by spores on the surface was approximately 30 microns.

Subsequent growth, however, was more rapid on the surface, where the sporidial germ tubes grew to a length of from 150 to 180 microns before any change was observed in the sporidia produced by the totally submerged spores.

SOME MORPHOLOGIC FEATURES OF THE GERMINATION OF SPORES OF *UROCYSTIS TRITICI*

McAlpine (3) has described the spore form and also some of the features of spore germination. On germination each spore of the spore ball may produce a promycelium. Frequent splitting of the spore wall was observed (Figs. 1, a, c, d, and f), although this was not noted in all cases. Under normal conditions the promycelium usually attains dimensions of about 20 to 30 by 5 μ , although in one instance the promycelium grew to a length of 70 μ before forming sporidia (Fig. 1, k and Fig. 2, o); then at the tip there are formed a number of small protuberances (Fig. 1, b and pl. X, fig. 1), which elongate to form a whorl of from 1 to 6 sporidia, 2 to 4 being most frequently found. The sporidia are somewhat cylindrical in shape, and, when fully formed, are usually about 30 by 5 μ in size. At first the young sporidia remain aggregated at the tip of the promycelium, but, as growth proceeds, they gradually diverge to form a series of finger-like protuberances. Further growth most frequently is manifested by a prolongation of the sporidium while still attached to the promycelium (Fig. 1, j and l). Sometimes additional "buds" may be formed at the tip of the promycelium at the base of the whorl of sporidia (Fig. 1, c, g, h); and these may be prolonged into germ tubes before any are produced by the first formed sporidia (Fig. 1, 1).

These prolongations, which may be considered as germ tubes or infection threads, have been observed to grow to a length of about 900 μ . They are fairly constantly about 2 or 3 microns wide. The protoplasm becomes concentrated at the tips, while, at the same time, the remainder of the mycelium may become multicellular (Figs. 1, j and 2, o). When the septa are formed, however, the cells are devoid of protoplasm.

Fusion of sporidia was noted several times (Fig. 1, d, e, f, and n); and occasionally there also was branching and fusion of the germ tubes (Fig. 1, i).

Under some conditions of germination other irregularities were observed. Occasionally no sporidia were developed, but the promycelium

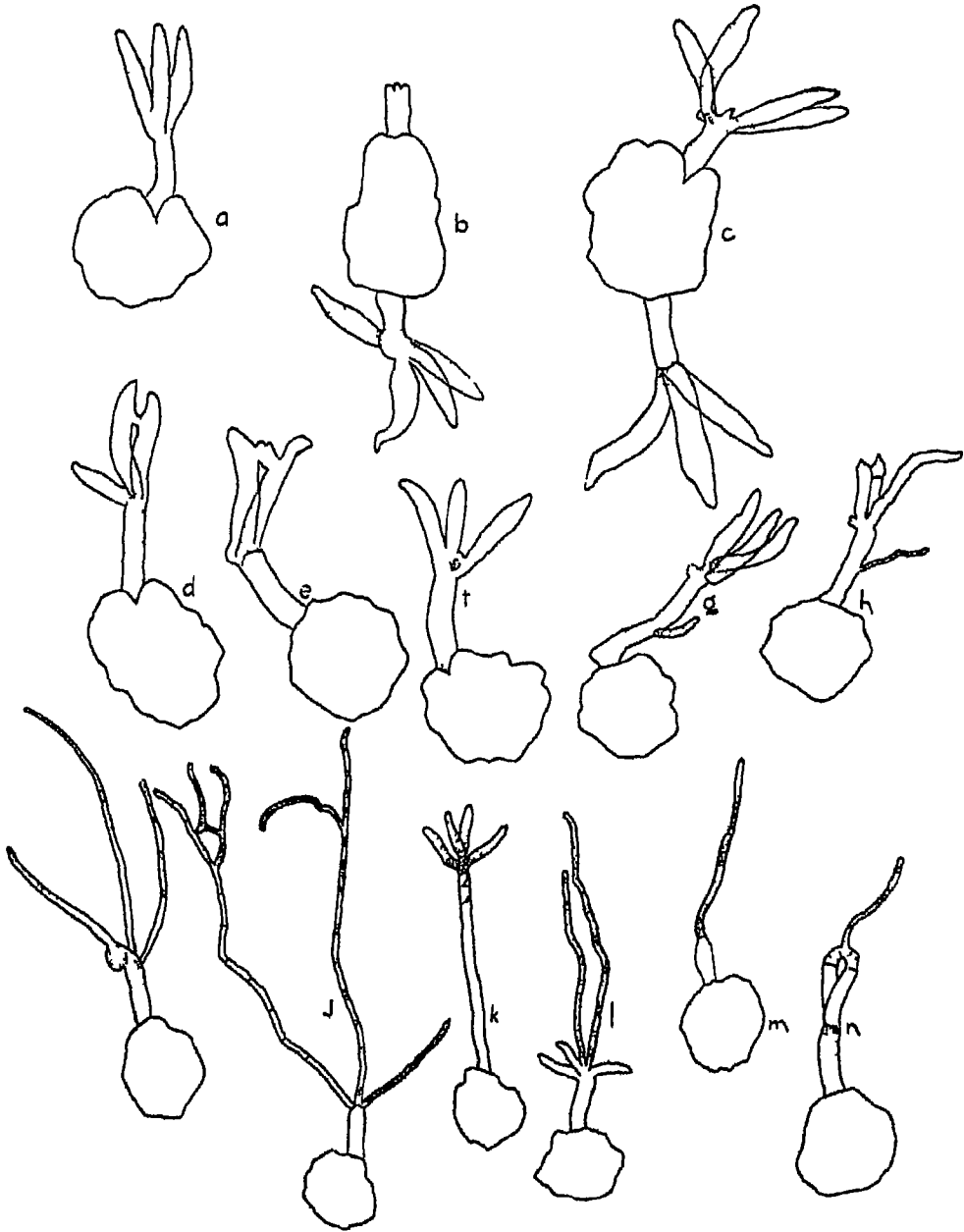


FIG. 1. Germination of spores of *Urocystis tritici*: a-f, $\times 450$; g-h, $\times 330$, i-n, $\times 250$. (Camera lucida drawings.)

developed into a germ tube (Figs. 1, m and 2, q and u). In one case a secondary promycelium was produced instead of a sporidium. This grew to a length of about $60\ \mu$ and then produced a number of protuberances which may be considered as sporidia (Fig. 2, p).

Sometimes particularly when spores germinate at high temperatures promycelia were formed but no normal sporidia were produced the

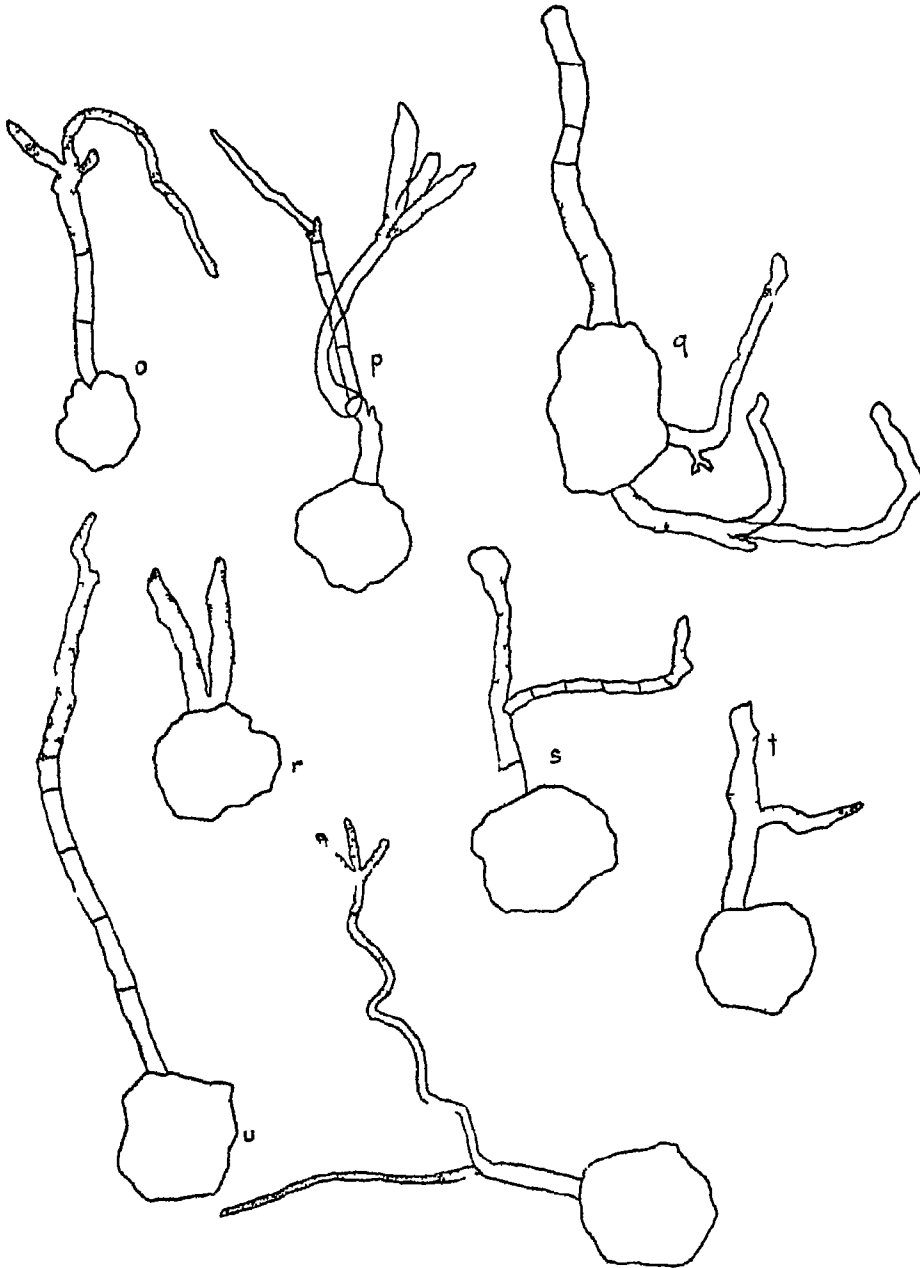


FIG. 2 Germination of spores of *Urocystis tritici*. Less normal type o, p, r, s, t, v $\times 330$; q, u $\times 450$. (Camera lucida drawings.)

mycelium merely branched in an irregular manner (Fig. 2 s and t). Several times a portion of the protoplasm was cut off in an offshoot from the promycelium (Fig. 1, g and h).

Thus it is seen that there may be considerable variation on the character of the germination of the spores of *Urocystis tritici*.

DISCUSSION AND CONCLUSIONS

The first prerequisite to the development of a disease is the inoculum. It is very evident, however, that susceptible plants may be inoculated with abundant inoculum but yet infection may not occur.

This fact often has been noted with respect to flag smut of wheat. Even though soil may be heavily infested with the spores of the causal organism, a clean crop sometimes may be grown. Under other conditions, however, a relatively small amount of inoculum may be sufficient to cause a serious outbreak of the disease.

The spores of *Urocystis tritici* have been known to germinate capriciously. In fact, it has been difficult to make pathogenicity studies on account of the erratic behavior of the spores.

It has sometimes seemed almost impossible to reconcile the abundant development of smut in the field with the apparent lack of viability of spores. Soil temperature, soil moisture and aeration are known to affect greatly the development of some smut fungi. But the evidence obtained from field observations and from spore germination studies seemed to indicate that these factors alone were relatively unimportant in influencing the infection of wheat by *U. tritici*.

It seemed, therefore, that a sequence of circumstances occurred under natural conditions which were not duplicated in experimental trials. Many unsuccessful attempts were made to hit upon the right combination until it was found that something in plant tissues, and, to some extent in soil infusions, stimulated spore germination in a most remarkable manner. The stimulating substances must be present at the right time and in appropriate concentrations in order to produce the maximum effect. This is indicated particularly by the fact that they are not effective unless the spores have been presoaked. Neither are they effective when the spores have been in water too long nor when they have been kept at an unfavorable temperature. The substances are volatile and appear to be diffusible, at least from the rootlets of young wheat seedlings.

Soil moisture, soil temperature, soil aeration, and the presence of a stimulatory substance at any given time may not be necessarily conducive to infection. It would appear that these factors must operate in the proper order and must be correlated chronologically. Sometimes the factors may operate together in nature in such a manner as to cause the development of flag smut epidemics.

Since the spores of *U. tritici* may retain their vitality in the soil for several years, it is possible that they may be stimulated to germinate by suitable cultural methods; the incorporation of some non-susceptible crop of the right kind in the rotation system, and the practice of "green manuring" might be expected materially to decrease the amount of inoculum in the soil between successive wheat crops.

It is desirable that further studies be made, especially on the factors governing spore germination in the field. Time of planting is frequently of great importance under Australian conditions. Further information on the temperature-moisture relationships under field conditions might reasonably be expected to yield data which would suggest further modification of present cultural practices.

SUMMARY

1. Extensive spore germination studies were made on the spores of *Urocystis tritici* Koern. Practically no spores germinated in ordinary nutrient solutions. At the end of from 4 to 16 days usually only a few spores had germinated in distilled water. Changes in hydrogen-ion concentration and in surface tensions of media appeared in themselves to be of little importance.

2. When small portions of young wheat plants were added to distilled water in which the spores had been presoaked for several days, from 70 to 95 per cent of the spores germinated.

3. Presoaked spores germinated when they were transferred to wheat plant infusions of appropriate dilution or to a distillate obtained from these infusions.

4. The stimulatory substances are volatile, but their exact nature has not been determined.

5. Young tissues of other cereals and a number of other plants also stimulated spore germination.

6. Uninjured wheat seedlings stimulated germination of presoaked spores, as well as did the cut tissues.

7. After spores had been soaked in water for 28 days, they still responded to some extent to stimulating substances.

8. The minimum temperature for spore germination apparently is about 5° C. The germ tubes, however, will grow at 0° C. The maximum temperature appears to be a little less than 32° C. The optimum temperature for spore germination appears to be between 18 and 24° C.

9. The effect of temperature on spore germination varies with the previous treatment of the spores. When a stimulating substance is

added to spores which have been presoaked for 6 days at 20° C the optimum temperature for growth of the germ tubes is about 24° C.

10. Spores which have not been presoaked germinate most quickly at about 18° C. When they are kept at temperatures of over 24° C. for a considerable length of time, they respond less readily to substances which stimulate germination.

11. After total immersion in distilled water for several days at 20° C, the spores germinated readily on the addition of plant tissue. Growth of promycelia was slightly slower than at the surface of the medium.

12. Cultural practices may result in germination of spores in the soil, in a manner similar to that indicated in laboratory experiments.

13. There may be considerable variation in the morphologic features of the promycelia and of the sporidia, according to the conditions under which germination occurs.

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AN IMPORTANT PERIOD IN THE LIFE HISTORY OF TWO BACTERIAL ORGANISMS CAUSING LEAF-SPOTS ON TOBACCO.

W. D. VALLEAU.

WITH ONE FIGURE IN THE TEXT

Angular leaf-spot and wildfire of tobacco are two leaf-spot diseases which have been of considerable economic importance in Kentucky since 1920. In 1920 and 1921, and to a less extent in 1922, these bacterial diseases caused much damage in plant beds and serious losses in the field.

Observations of numerous diseased plant beds and fields indicate that plant bed infection practically always precedes field infection and that disease-free plant beds generally produce disease-free plants in the field. Control of the diseases, therefore, depends on their elimination from the plant bed. The various sources of plant bed infection are not yet definitely known but the work of Fromme¹ leaves little doubt that the organisms may enter the plant beds on the seed. It is also likely that they may be introduced on the feet of laborers who have been working in infected tobacco trash, as Clinton and McCormick² have shown that the wildfire organism may live over winter in cured tobacco. We have some evidence that the angular leaf-spot organism, at least, may live in the soil for considerable periods of time.

Because of the numerous possible sources of infection, seed treatment as suggested by Fromme cannot be expected to be efficient except under well controlled conditions. These are hard to obtain when the preparation of the beds is left to untrained help. As a consequence, it is desirable that methods of control be devised which will prevent development of the organisms in the plant bed entirely, no matter how introduced, and thus completely prevent plant infection.

Spraying and dusting with various copper compounds³ have been suggested as means of control and appear to be effective to a considerable

¹ Fromme, F. D. and S. A. Wingard. Treatment of tobacco seed and suggested program for control of wildfire and angular-spot. (Abstract) *Phytopath.* 11: 48-49. 1921.

² Clinton, G. P. and F. A. McCormick. Wildfire of tobacco in Connecticut. *Connecticut Agric. Exp. Sta. Bull.* 239: 365-423. *Pl.* 29-32. 1922. Literature, p. 422-423.

³ Chapman, G. H. and P. J. Anderson. Tobacco wildfire. *Massachusetts Agric. Exp. Sta. Bull.* 203: 67-81. *Pl.* 1. 1921.

degree at least. In order to be entirely satisfactory, control must be complete, as the introduction into the field of only a few plants infected with either the wildfire or angular leaf-spot organisms appears to be sufficient, if weather conditions are favorable to spread, to infect large plantings. In other words, the treatment should prevent absolutely the appearance of the diseases on the plants. This necessitates a more accurate knowledge of the life history of these organisms in the plant bed.

The purpose of this note is to record the observations of the writer on the method of primary infection of young tobacco plants in the plant bed and its bearing on the life history and control of these two leaf-spot diseases.

During the past three years the writer has observed numerous cases of wildfire and angular leaf-spot in tobacco plant beds and has had occasion to study the process of infection of young plants. These observations have given little or no information as to the original source of the organisms in the beds, but it may fairly be assumed that they were either present at the time the seed was planted or were introduced at that time, as in Kentucky the beds are hardly touched between the time of sowing and the first appearance of the diseases.

The first infections nearly always occursimultaneously on a considerable number of plants in a small area in a bed or possibly in several similar areas in the bed. They usually appear when the plants are about the size of a quarter. At this time the largest leaves have spread out enough so that their tips just come in contact with the moist soil. It is at this point of contact that infection occurs.¹ These observations suggested that the causal organism was introduced into the soil in spots, possibly on a seed or a small piece of tobacco trash, or in some other way, and that the bacteria rapidly multiplied in the soil and gradually spread out in more or less of a circle during the time when the plants were developing to a size where infection would be possible. As soon as the tips of the leaves come more or less permanently in contact with the infected soil they become diseased, resulting in a rapid soft rot of the tip portion of the leaf. From this time on, secondary infection seems to be purely the result of mechanical conditions—splashing, insects, movements of leaves, etc. The organisms may be readily spread over the remainder of the bed by flowing water.

As the primary plant infections seem always to result from contact of the small leaves with diseased soil, it was thought that control might

¹ Clinton and McCormick (l. c.) have figured and described this stage of the wild fire disease on young plants.

be effected through the addition of toxic materials to the soil early enough to prevent completely multiplication of the organisms in the soil. Consequently an experiment was planned in which sulfur was to be used as the toxic agent. A plant bed was prepared on October 23, 1921, in the greenhouse. Four plots were laid off in this bed. The first received no sulfur, the second sulfur at the rate of 500 pounds per acre, the third at the rate of 1000 pounds and the fourth at the rate of 2000 pounds per acre. Flowers of sulfur was used. It was thoroughly mixed with the upper $\frac{1}{4}$ to $\frac{1}{2}$ inch of soil *before* the seed was sown. The seed used was a mixed lot of Burley seed obtained by recleaning the waste seed carried over in the process of cleaning seeds for numerous growers. If the seed carries infection, such a lot would be expected to be diseased and so it proved to be. This seed was sown in the four plots. Two other plots were planted in the same bed. One was sown from seed which had been obtained from a commercial seed grower who did not practice bagging the seed heads, thus exposing the pods to infection. The other plot was planted with a root-rot resistant strain of White Burley from a bagged seed head.

Germination proceeded normally in all the plots. After the seedlings were well established but before the production of the first true leaf, some plants in each of the plots damped off. The number of plants which died was in direct proportion to the amount of sulfur in the beds. In those with none, a few plants died. In the bed treated at the rate of 500 pounds per acre, the stand was reduced to 75 plants; where 1000 pounds was used, 25 plants lived, whereas in the plot treated at the rate of 2000 pounds, only 7 plants remained alive.¹ In the untreated control plot about 200 plants were present. The stand in plants from commercial seed was about $\frac{1}{3}$ that of the control plot and about identical with that in the 500 pound plot. The various plots were separated from one another by ordinary laths set on edge and buried about half way in the soil. This was not sufficient to prevent some splashing from plot to plot while watering and no particular care was used in this regard.

• The first bacterial spots were noticed on December 21, 1921. At that time diseased plants were found in the untreated control plot and in the untreated plot planted with commercial seed. No infected leaves were

¹ The effect on soil micro-organisms was also very striking in the treated plots. In the untreated plots the green and blue-green algae common in greenhouses formed a thin mat over the surface of the soil while they were completely inhibited in the treated plots with the exception of two or three small spots not over two inches in diameter where some growth took place. In figure 1, this effect can be clearly seen in the different color of the soil of the three plots shown.

found in the three treated plots or in the plot planted from bagged seed. The plots were kept under observation until January 12, 1922. Not a single suspicious spot was found in any of the three treated plots, in spite of the fact that there was constantly a chance of the organisms being washed in from the neighboring diseased plots. The toxic action of the sulfur continued to protect these plots from soil infection. Toward

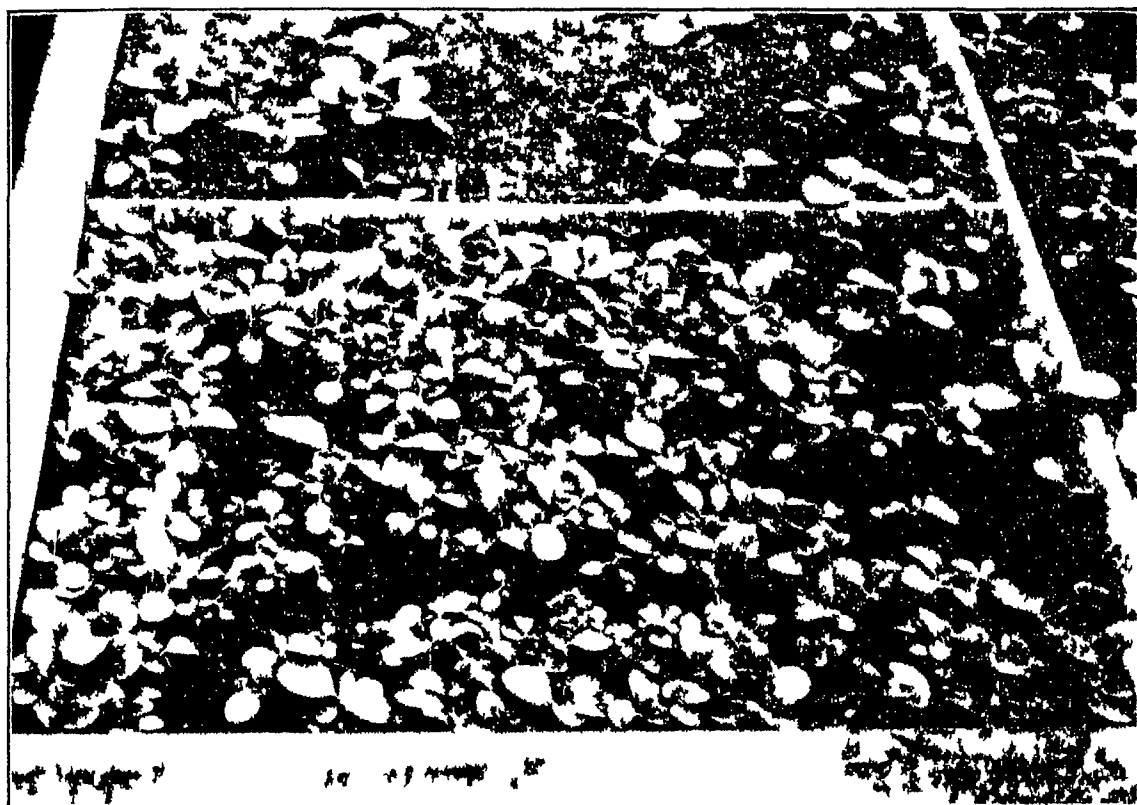


FIG. 1. Photograph taken December 27, 1921, of the untreated control plot showing numerous diseased leaves in contact with the soil. In the rear a portion of the plot treated with sulfur at the rate of 500 lbs. per acre. At the right a portion of the untreated plot planted with commercial seed. A few diseased plants may be seen in it.

the end of the period of observation a few spots had appeared on the plants grown from bagged seeds but bacteria were not demonstrated in any of those examined. Apparently the bagged seed were free from infection when sown and the soil in this plot, therefore, remained undiseased at least until the plants were beyond a stage where leaf-tip infection could readily occur. The diseased leaves in the two infected plots became more numerous, making the plots conspicuous because of the unhealthy appearance of the plants.

The relation between the primary leaf infections and soil infection was very marked in the two diseased plots. The infections could readily

be traced to contact of the first, second, third or fourth leaf tips with the surface of the soil. In the treated plots, although the leaves were as frequently in contact with the soil as in the other plots, no infection occurred and the leaves remained healthy (Fig. 1). In the two diseased plots, the plants at one time were nearly defoliated, but later as new leaves were produced which were more erect, the plants gradually assumed a healthier appearance as is shown in figure 1.

Because of the injury to the stand in the treated plots, the writer does not recommend sulfur as a means of controlling these diseases in the seed bed, but the fact seems to be established that soil infection occurs first and leaf infection follows, and that complete control of the diseases may be obtained by applications of toxic materials early enough to prevent multiplication of the organisms in the soil.

KENTUCKY AGRICULTURAL EXPERIMENT STATION.

PHYTOPATHOLOGICAL NOTES

Sereh disease of sugar cane in Singapore. In the Singapore Botanic Gardens on September 19, 1922, the writer observed sugar cane with shortened internodes and with abnormal development of aerial roots from the nodes. On cutting into the cane, short red streaks were observed at the nodes. The leaves of the affected cane were not streaked or wilting as is the case with cane affected with the Java gum disease. The disease was similar in every way to the sereh disease of Java. Only one cane stool was found to be clearly affected.

Inquiry developed that the canes for this field had been introduced from Java.

To the writer's knowledge this is the first definite observation of the occurrence of sereh outside of Java, although it is known that Java canes have been introduced into Formosa and the Argentine Republic. It is evident that there are still a few cane breeders and sugar producers who are not aware of the risks which they run in importing canes from the oriental countries; in addition to sereh, Fiji disease, downy mildew and cane smut are destructive diseases of sugar cane in the Orient which are not general in the western hemisphere as yet.

Fiji disease especially is a devastating disease under many conditions, equal in effect to such diseases as chestnut blight, pear blight, or pineapple wilt.

The possible introduction of these diseases into the western hemisphere would mean not only financial losses to American sugar interests but ultimately increased cost of sugar to the American consumer.—H. ATHERTON LEE.

Mottle-necrosis of sweet potatoes. For several years the writers have collected and received specimens of sweet potatoes from various parts of the United States which showed a brown, irregularly formed discoloration of the sweet potato as shown in cross section in figure 1. This mottling appearance characteristic of this disease has been observed at digging time. The patches of dead chocolate-brown tissue are more or less irregularly located throughout the potato. Some of the patches appear to be completely isolated, that is, they have no connection with any of the other dead areas in the potato. In mild cases of this trouble there is no evidence on the surface of any internal disorder. However, in more severe or advanced cases of the disease there are dead areas at the surface. These dead surface spots may occur at the end or at any

point on the surface between the two ends (Fig. 1). A series of cross sections show that necrotic tissue may be found in any part of the potato.

The exact cause of mottle-necrosis of the sweet potato is not known. It was especially bad in the vicinity of Washington, D. C., in the fall of 1922 on the Triumph variety. A less amount was found on the Little Stem and Big Stem Jerseys.

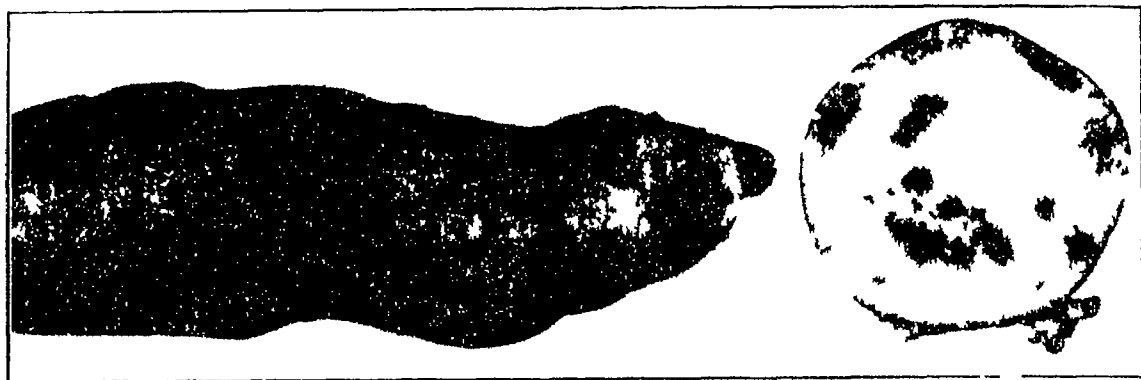


FIG. 1. Mottle-necrosis of sweet potatoes. Surface and cross section views at left and right respectively.

A large number of plantings in agar plates were made from the dead spots. In most cases no organism was obtained from these plantings. A few gave bacteria, a species of *Penicillium* and one or two species of *Fusarium*. Plate plantings from specimens collected by one of the authors collected in North Carolina in 1918 gave *Fusarium oxysporum*. Inoculation experiments with this organism gave negative results, however. So far the writers have been unable to demonstrate the presence of any mycelium in the necrotic tissue in isolated spots within the potato. Fungi, however, have been found in the dead tissue in contact with or near to the surface. This disease is being further investigated by the writers.—L. I. HARPER, J. I. LAURITZEN, J. L. WEIMER.

Internal break down of sweet potatoes. Each year the writers have received and collected sweet potatoes which are more or less pithy. A cross section shows that many of the cells of the potato have been pulled apart. The resulting cavities give a more or less cottony appearance to the potato. There is little or no discoloration of this tissue. Cultures from such tissue have always given negative results.

A microscopic examination of such material shows that much of the starch in the vicinity of the ruptured cells has been used up. Internal break down is most prevalent and conspicuous after the potatoes have been several weeks in storage. Some varieties seem to be more suscep-

ible than others. The trouble seems to be associated with conditions of storage. It is more prevalent when the house has been kept fairly dry.—L. L. HARBER, J. I. LAURITZEN, J. I. WEIMER.

Lightning injury to tomatoe. An interesting case of lightning injury was found in an experimental tomato field of the Purdue University Agricultural Experiment Station near LaFayette Ind. on July 7, the second day after a severe electrical storm. Evidence that the lightning had struck in this vicinity was furnished by the occupants of a farm house at one side of this field. The injury to the tomatoes occurred in a more or less circular area about forty feet in diameter. The plants at the center were killed and the severity of the injury became less toward

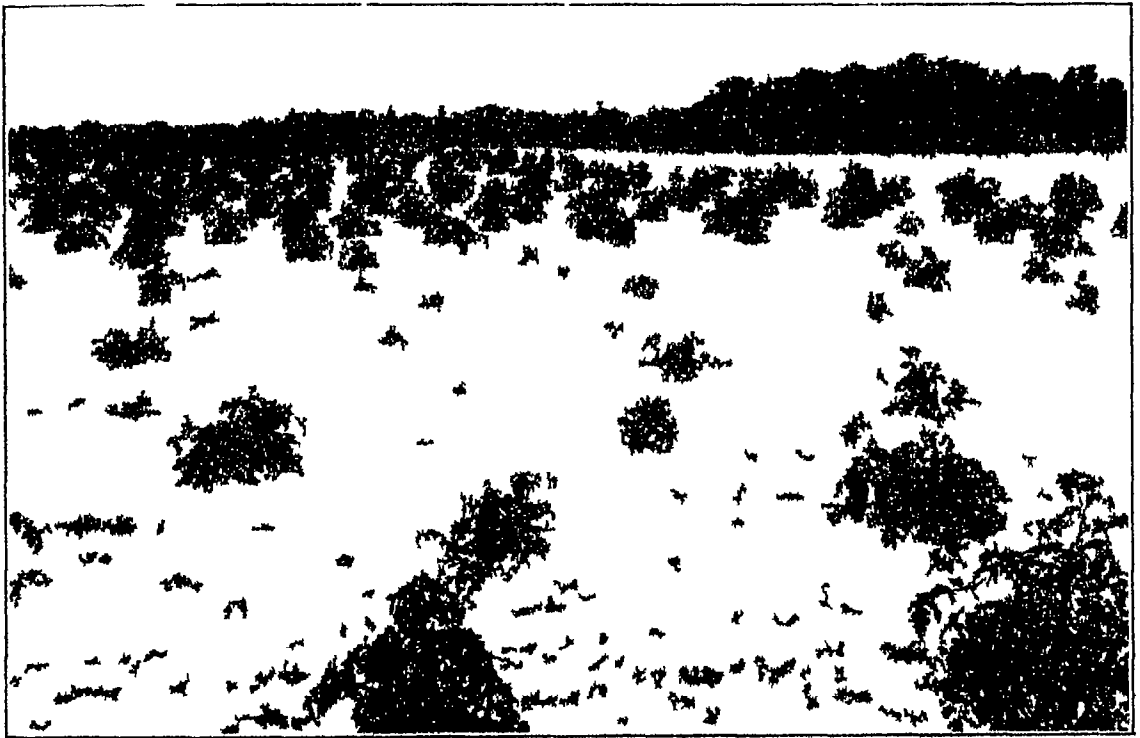


Fig. 1. Lightning injury to tomatoes.

the outer limits of the area. The interesting feature was that the injured plants showed among other symptoms typical hollow stem, a condition characterized by the collapse of the epith in the stem.—H. D. BROWN AND MAX W. GARDNER.

An international conference of phytopathologists and economic entomologists will be held in Holland from June 25 to 30, 1923. Those wishing to attend are urged to arrange their trip so as to be at Wageningen on June 24. The committee with Dr. H. M. Quanjier, president de-

sires that those wishing to read papers send a short abstract to the secretary, Mr. T. A. C. Schoevers, Villapark 8, Wageningen.

The committee has arranged the following provisional program:

Monday, June 25. Presidential address of welcome and lecture on plant diseases. Demonstration of experiments, especially on potato diseases.

Tuesday, June 26. Address by Mr. van Poeteren on organization and methods of the Dutch Phytopathological Service.

Wednesday, June 27. Field trips.

Friday, June 29. Meeting at Baarn. Address by Prof. Westerdijk on the cultivation of parasitic fungi and demonstration of research. Discussion and inspection of the international collection of fungi cultures.

Saturday, June 30. The Hague. Informal meeting at the office of the Minister of Agriculture.

English will be the official language of the conference.

The committee in charge consists of Professor Westerdijk, Mr. v. Poeteren, and Dr. v. Slogteren in addition to Dr. Quanjer.

A cordial invitation is extended to Americans. Detailed announcement may be expected later and meanwhile any inquiries may be addressed to Dr. H. M. Quanjer, Head of the Institute for Phytopathology at Wageningen, Holland.

Notice has just been circulated to the effect that Saccardo's "Sylloge Fungorum" is to be continued under the direction of Dr. Montemartini. The first volume of the Sylloge Fungorum was issued in 1882. Since then 22 volumes have been published. Two more volumes have been completed by I. B. Traverso and A. Trotter and are now ready for publication. It is requested that publications, especially those on mycology, be sent to the Laboratorio Crittogamico Italiano, Pavia, Italy.

Officers for the western division of the American Phytopathological society, which met in Salt Lake City, were elected for the coming biennium as follows:

President, S. M. Zeller, O. A. C., Corvallis, Oregon; Vice President, J. P. Bennett, University of California, Berkeley, Calif.; Secretary-Treasurer, C. W. Hungford, University of Idaho, Moscow, Idaho; Councillor, representing the Pacific Division, F. D. Heald, Washington State College, Pullman, Washington.

Personals.—Dr. H. M. Jennison, for several years assistant professor of botany at the Montana Agricultural College, Bozeman, has accepted

a position as associate professor of botany in the University of Tennessee, Knoxville. He transferred to his new position September 15.

Prof. G. R. Bisby, Professor of Plant Pathology at Manitoba Agricultural College, has returned to Winnipeg after a year spent at the Imperial Bureau of Mycology, Kew, England, where he has been associated with Dr. F. J. Butler.

Dr. G. R. Lyman, plant pathologist in charge of the Plant Disease Survey of the U. S. Department of Agriculture, has been appointed Dean of the College of Agriculture of West Virginia University, Morgantown, West Virginia, where he will have supervision of the three divisions of agricultural work of that institution, which include the resident instruction in the College of Agriculture, and the work of the Agricultural Experiment Station, and of the Extension Service. Dr. Lyman will enter upon his new duties on January 16, 1923.

Mr. Philip Brierley of St. Paul, Minn., a graduate of the New Hampshire State College, and the University of Minnesota, has been appointed Junior Plant Pathologist, effective July first. He will have headquarters in Washington and will assist Mr. Freeman Weiss in the investigations of wart disease of potatoes. Mr. Brierley has been employed previously by this Bureau for temporary periods in connection with work on blister rust control.

THE FOURTH ANNUAL SUMMER FIELD MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The fourth of the series of annual field conferences of the American Phytopathological Society was held in 1922, in the important trucking sections of southern Delaware, New Jersey, and near Philadelphia, from August 29 to September 1. The convention was given over primarily to the study of diseases of vegetables, the other three annual summer meetings of the Society, held in 1919, 1920, and 1921, being planned with especial reference to potatoes, fruits and cereals, respectively.

The members of the conference gathered at Seaford, Delaware, on the afternoon and evening of August 28, and the next day Dr. J. F. Adams, of the Delaware Experiment Station, conducted the party on a field trip in the vicinity of Seaford. The new and up-to-date sweet potato storage house of the Johnson Potato Storage Company was inspected. This is probably the largest sweet potato house in the country, it having a capacity of 125,000 five-eighths bushel baskets. Other stops were made at farms where crops of cantaloupes, tomatoes, asparagus, cowpeas, soy beans, and especially sweet potatoes were seen. Of the sweet potato diseases, white rust (*Albugo ipomoeae-panduranae*) was very conspicuous and causing more damage than is usually the case with this disease. Fusarium wilt (*Fusarium batatas* and *F. hyperoxysporum*), black rot (*Sphaeronema fimbriatum*), and leaf spot (*Septoria bataticola*) were seen, and extreme fasciation of the stems of sweet potatoes was very common in many fields. In the various sweet potato fields visited, the members had the opportunity to observe the influence of seed treatment and crop rotation on disease prevalence, as well as to note results being obtained from the use of different varieties and fertilizers. An experimental field of cantaloupes, badly damaged by hail and downy mildew (*Pseudoperonospora cubensis*) was observed. Downy mildew was very severe in Delaware this season, at least 50 per cent of the crop being lost, according to Dr. Adams. In tomatoes *Septoria* leaf spot (*Septoria lycopersici*), Fusarium wilt (*Fusarium lycopersici*), and mosaic (undetermined) were common. The tomatoes of the vicinity were in rather poor condition owing to wet weather and disease. Diseases of other crops seen were asparagus rust (*Puccinia asparagi*) and the leaf spots of cowpeas caused by *Cercospora cruenta* and *Amerosporium oeconomicum*. A visit was made to a large canning factory at Seaford, where an average of 75,000 cases of peas and 100,000 cases of catsup, chili sauce, and tomatoes are packed annually. Following this, the party left for Philadelphia.

The next day, August 30, was "New Jersey Day" with the program in charge of Dr. Mel. T. Cook. Automobiles conveyed the party from Camden to Glassboro, stopping at numerous points on the way. On the farms of the Campbell Soup Company near Riverton, experiments for the control of *Septoria* leaf spot and other diseases of tomatoes by the use of Bordeaux mixture and anhydrous copper dust were inspected. At Mt. Laurel, sweet potato varieties grown on soil heavily infested with Fusarium were being compared for resistance to Fusarium wilt, and the relation of the prevalence of this disease on soil type and crop rotation was being demonstrated. One of the most interesting stops of the morning was at Marlton, where in a large plantation of okra the wilt caused by *Verticillium albo-atrum*, and certain leaf spot diseases of this crop were observed. The *Verticillium* was very abundant, affecting many plants and cutting down the yield to a great extent. Inadequate crop rotation in recent years has evidently favored the increase of the disease. At Glassboro, the Repp Brothers

Company cold storage plant and orchards were inspected. In part of the latter the Insecticide and Fungicide Board of the U. S. Department of Agriculture was conducting some of its official tests with various commercial fungicides, and the effects of some of these classes of spray materials on trees were noted.

On the evening of August 30, specialists of the U. S. Department of Agriculture spoke on the subject of marketing and market pathology. Mr. F. G. Robb, of the Bureau of Markets, discussed the various ways by which fruits and vegetables pass from the producer to the consumer, and explained the operation of the fruit and vegetable inspection service that has been conducted by the Bureau of Markets for the past five years. Mr. F. C. Meier of the Bureau of Plant Industry in his talk which was illustrated with a number of beautifully prepared lantern slides, mentioned the losses from decay that are incurred between the field and the market. He told of the methods of training the market inspectors to distinguish between the various decays and discussed some of the important problems that arise in connection with the work. Dr. L. L. Harter of the Bureau of Plant Industry in his paper on "Research problems in plant pathology and their relation to the transportation and marketing of perishable vegetables," brought to the attention of the conference some of the research that already has been done on the relation of storage conditions to the development of decay-producing organisms. He showed the need for further investigation of this kind, and emphasized the great desirability of further cooperation between the plant pathologist and the market investigator.

August 31, "Market Pathology Day," which was spent entirely in Philadelphia, was full of interest. Under the leadership of F. C. Meier the party visited the Pennsylvania Cold Storage and Market House at 30th and Chestnut Streets; the Dock Street Wholesale Market; the auction piers of the Pennsylvania and Baltimore and Ohio railroads, located at piers 11-15 on the water front; the auction market operated in connection with the piers; the Philadelphia offices of the Bureau of Markets of the U. S. Department of Agriculture; and the Reading Terminal Market and cold storage rooms. This series of inspections gave the members of the conference an excellent idea of the complicated process of marketing fruits and vegetables from the time they come into the city until they reach the hands of the consumer. They were also able to familiarize themselves with the important work that is being done by the Bureau of Markets of the U. S. Department of Agriculture.

"Bustleton Day," September 1, was in charge of Dr. W. S. Beach of the Pennsylvania Agricultural Experiment Station. In the automobile trip through the extensive trucking sections near Bustleton, a great variety of vegetable crops were seen,—celery, rhubarb, spinach, lettuce, Lima bean, cantaloupes, horse-radish, parsley, tomatoes, cabbage, and sweet corn, as well as numerous other truck crops being grown there in large quantities. On account of this diversity of crops a large number of diseases were to be seen. Among the commonest were late blight (*Septoria petroselinii*) and early blight (*Cercospora apii*) of celery; crown rot (*Phytophthora cactorum*), leaf spot (*Phyllosticta*) and anthracnose (*Colletotrichum*) of rhubarb; pod blight (*Diaporthe phaseolorum*), downy mildew (*Phytophthora phascoli*) and other pod and leaf spots of the Lima bean; black rot (*Bacterium campestris*) and soft rot (*Bacillus carotovorus*) of cabbage and lettuce drop (*Sclerotinia* spp.). A visit was made to the Research Laboratory of the Pennsylvania Agricultural Experiment Station at Bustleton where Dr. Beach explained the work that is being conducted. Lettuce plants artificially inoculated with *Sclerotinia minor*, and rhubarb infected with different species of *Phytophthora*, were of especial interest at the laboratory. Experiments for the control of celery leaf diseases

(*Septoria ptyosclini* and *Circospora apii*), downy mildew of Lima bean (*Phytophthora phaseoli*), lettuce drop (*Sclerotinia minor* and *S. libertiana*), and rhubarb crown rot (*Phytophthora cactorum*) were inspected.

As was the case with the other annual field conferences, the society was fortunate in having present a number of plant pathologists and botanists from other countries. Dr. I. B. Pole-Evans, Chief of the Division of Botany, Department of Agriculture, Pretoria, Union of South Africa, and Senor Alfonso Madariaga, of the Department of Agriculture of Mexico, were officially designated by their governments to come to this country for the purpose of attending the conference. Other visitors of note were Dr. Seiya Ito, Hokkaido Imperial University, Sapporo, Japan, Dr. Takowo Hemmi, Kyoto Imperial University, Kyoto, Japan, Dr. Rudolph Kuraz of the Czechoslovakian Legation, Washington, D. C., Mr. Carlos A. Vallejo of the Argentine Embassy, Washington, D. C., Mr. R. J. Noble of Australia, Mr. N. G. Teodoro, Department of Agriculture and Natural Resources, Manila, Philippine Islands, Mr. G. O. Ocfemia, College of Agriculture, Los Banos, Philippine Islands, Dr. C. Boyle of the Department of Agriculture and Technical Instruction for Ireland, and Mr. D. Borodin of the Russian Agricultural Bureau, New York City. The meeting was full of interest and those who attended, between sixty and seventy-five in number at various times, found it an extremely profitable occasion. The pathologists of Delaware, New Jersey, and Pennsylvania, who had the program in charge, the local growers, county agents, and others, who assisted by furnishing automobile transportation and in perfecting arrangements, the officials of the Pennsylvania Railroad, the Reading Terminal Market, and the U. S. Bureau of Agricultural Economics, who rendered valuable service on Market Pathology Day, all deserve much credit, and have the thanks of the entire Society for making the meeting such a complete success.—R. J. HASKELL, Secretary, Advisory Board, American Plant Pathologists.

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EXPERIMENTS WITH HOT WATER, FORMALDEHYDE, COPPER CARBONATE, AND CHLOROPHOL FOR THE CONTROL OF BARLEY SMUTS

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INTRODUCTION

The smuts of barley, loose smut caused by *Ustilago nuda* (Jens.) Kell. and Sw., and covered smut caused by *Ustilago hordei* (Pers.) Kell. and Sw., cause considerable annual loss to the barley crop of the United States. According to authoritative investigations the loose smut of barley behaves very much like the loose smut of wheat. It will not be necessary to give a history of these investigations here. An adequate discussion is given by Freeman and Johnson (4) who also report the results of their own studies which confirm the results of previous investigators. The loose smut infects the grain at flowering time or soon afterwards. The spores from smutty heads lodge within the glumes of normal heads when the glumes are open at the flowering period. These spores germinate and infect the young ovaries immediately or before they are one-third grown. The fungus remains within the seeds through maturity and until they are sown, when it revives from its state of dormancy as the seeds germinate and grows up through the young plants, causing the abnormal or smutty heads.

Covered smut of barley is similar to bunt of wheat and the smuts of oats, so far as infection is concerned. The smut spores are carried externally or within the glumes on seed from infested fields. Infection takes place through the coleoptile of the young seedlings shortly after the seeds germinate. The fungus grows up through the plant and produces smutty heads. A description of the symptoms of the barley smuts will not be given, due to the fact that they have been adequately described by various authors.

The purpose of this paper is to discuss the results obtained by the use of various seed treatments for the control of these barley smuts. Special consideration has been given the formaldehyde and modified hot-water treatments. The modified hot-water method has been consistently recommended for the control of the loose smuts of barley and

wheat. Jensen (7) devised and used this method in Denmark in 1887; two years later Kellerman and Swingle (9) recommended its use for the control of oat smut in the United States. For the control of covered smut of barley the formaldehyde or other methods of surface sterilization have been recommended. Bolley (2) in 1897 was the first in the United States to recommend the use of formaldehyde for the disinfection of seed grain. Many methods for applying these treatments have been devised since they were introduced. The hot-water method generally has been recommended where the seed is known to carry both loose and covered smuts.

PRESENT INVESTIGATIONS

Modified Hot Water and Formaldehyde

With the hopes of ascertaining more decidedly the limitations of the efficiency of the modified hot-water and formaldehyde treatments for the control of the barley smuts a series of studies was begun by the Office of Cereal Investigations, United States Department of Agriculture, at Arlington Farm, Virginia, in the fall of 1919. Numerous methods of applying hot water and formaldehyde were employed and the treated seed was sown in a rod-row series. At the same time, heavily smutted seed of three varieties of barley, Han River, Tennessee Winter, and Texas Winter, were sown in duplicate in one-fortieth acre plats after being treated as follows:

(1) Presoaked seed 4 to 6 hours in cold water and immersed for 10 minutes in water at 52° C. and spread to dry. A 13-minute dip in hot water (52° C.) generally is recommended (6, 8).

(2) Immersed seed 10 minutes in a formaldehyde solution containing 1 part of a 37- to 40-per cent formaldehyde solution in 320 parts of water, covered overnight and spread to dry. A longer period of soaking has been recommended where the seed is not covered before drying (6, 8).

(3) Sown without treatment.

The results obtained in the rod-row experiments were not sufficiently promising, when compared with methods in common use, to warrant a continuance of these tests after the second year, but the results secured in the one-fortieth acre plats proved to be of considerable interest, due to the fact that seed treated with formaldehyde produced plants as free from loose smut as did seed from the same source treated with hot water. Humphrey and Potter (6) in 1918 mentioned the fact that

¹ The writers are indebted to Dr. George M. Reed, formerly Pathologist in Charge of Cereal Smut Investigations, Office of Cereal Investigations, who outlined these studies and supervised the work in its earlier stages.

loose smut of barley is more or less controlled by the use of the formaldehyde treatment. In the fall of 1920 and 1921 five varieties were included in these experiments, as there seemed to be some varietal difference in the behavior toward these treatments. In the fall of 1920, however, hot water was used only on Tennessee Winter, the idea being to concentrate on the formaldehyde treatment. The sowings were made each year during the first week in October so as to insure the greatest possible uniformity as to time of sowing. All of the seed used in these experiments was grown on Arlington Farm, Rosslyn, Virginia, the season previous to sowing. The same methods of harvesting and thrashing were used in each case and the seed was stored in bags in the same shed until time for sowing. Table 1 shows the results of these experiments with the average yields in bushels per acre obtained in each case.

The averages given in table 1 show that formaldehyde was just as effective in controlling loose smut of barley as was the modified hot-water treatment, while it was less effective in most cases in controlling the covered smut, for which it has been recommended. As is further shown in this table, little was gained in the way of yields by the use of either treatment. Some of the averages show gains while others show losses, and on the whole the differences are about balanced.

The results in table 1 show very clearly that there is a varietal difference in the reaction of the barleys to these seed treatments, especially in so far as yields are concerned. This conclusion is considered justifiable in view of the fact that all the seed was grown on the same farm and handled in the same way previous to the treatment. In every case Tennessee Winter yielded better where the seed was treated, while the yields of Wisconsin Winter and Cusado were decreased without exception. Texas Winter was included in the experiment only one year and gave increased yields from both treatments. The yields of Greece and Han River were increased in some cases and decreased in others. Evidently the nature of the seed of the different varieties renders them more or less subject to injury by these treatments. These data lead us to conclude that different modifications of these treatments will be necessary for different varieties of barley, if seed injury is to be avoided. On the other hand, there seems to be little probability of obtaining satisfactory smut control by the use of less severe applications, as covered smut is not controlled satisfactorily by the methods used. These treatments appear to be satisfactory for Tennessee Winter, and, perhaps, for Texas Winter, but they would not be considered applicable to the other varieties employed in these tests.

TABLE 1.—Three years' data showing the comparative efficiency of the formaldehyde and modified hot-water seed treatment in the control of the loose and covered smuts of barley.

Variety	C. I. No.	Year of Crop	Results from treatment												Gain (+) or loss (—) in bu. per acre by treatment	
			Not treated			Formaldehyde			Hot Water							
			Per cent smut		Yield bu. per acre	Percent smut		Yield bu. per Acre	Per cent smut		Yield bu. per Acre		Formaldehyde	Hot water		
			Loose	Covered		Loose	Covered		Loose	Covered		Loose	Covered			
Tennessee Winter	257	1920	8.3	12.1	20.6	trace	trace	21.4	trace	trace	24.4	trace	+0.8	+3.8		
"	"	1921	11.7	21.9	20.9	0.3	3.4	26.7	0.0	trace	32.4	trace	+5.8	+11.5		
"	"	1922	1.1	3.3	47.6	0.1	2.5	52.5	0.2	1.54	48.9	trace	+4.9	+1.3		
Texas Winter	554	1920	18.0	33.0	13.7	0.0	trace	17.9	trace	trace	17.7	trace	+4.2	+4.0		
Han River	2163	1920	15.6	24.0	22.7	trace	trace	23.3	0.0	11.6	21.8	trace	+0.6	—0.9		
"	"	1921	9.6	32.0	22.9	0.15	26.7	30.4	—	—	—	—	+7.5	—		
"	"	1922	0.6	6.8	46.9	0.14	5.9	43.4	0.1	1.4	47.8	—	—3.5	+0.9		
Wisconsin Winter	2159	1921	10.9	8.3	27.6	0.0	2.4	26.4	—	—	—	—	—1.2	—		
"	"	1922	0.7	2.1	51.7	0.1	1.6	50.7	0.05	0.9	44.5	—	—1.0	—7.2		
Cusado	895	1921	14.6	28.7	22.1	0.0	13.8	20.5	—	—	—	—	—6.5	—13.6		
"	"	1922	3.7	7.5	51.5	trace	10.4	45.0	0.12	4.3	37.9	—	+4.8	—		
Greece	221	1921	6.3	18.8	21.8	0.0	7.3	26.6	—	—	—	—	+4.8	—		
"	"	1922	0.6	2.8	61.7	0.2	1.4	56.5	0.3	2.7	48.9	—	—5.2	—12.8		
Average for 5 varieties, Texas Winter not included	Comparing formaldehyde and untreated for 2 years (1921-1922)		6.0	13.2	37.5	0.1	7.5	37.9					+0.4			
Averages for 2 varieties, Tennessee Winter and Han River	Comparing formaldehyde, hot water, and untreated for 1 year (1922)		1.3	4.5	51.9	0.1	4.4	49.6	0.15	2.16	45.6		—2.3	—6.3		
	Comparing formaldehyde, and untreated for 3 years (1920-1922)		7.8	16.7	30.3	0.1	6.4	32.9					+2.6			
	Comparing formaldehyde, hot water and untreated for 2 years (1920 and 1922)		6.4	11.6	34.5	0.06	2.1	35.1	0.08	3.6	35.7		+0.6	+1.2		
Averages for Tennessee Winter for 3 years (1920-1922)			7.0	12.4	29.7	0.13	1.96	33.5	0.06	0.5	35.2		+3.8	+5.5		

Chlorophol and Copper Carbonate

In the fall of 1921, two other disinfectants, chlorophol, an organic mercury compound, and copper carbonate, were included in the agents used. Chlorophol is closely related to the mercuric compounds, Uspulun, which has been used in Germany since 1914 (11) or earlier, Germisan, of more recent discovery (1), and others of somewhat less value. These compounds have met with success in Germany where they are replacing the formaldehyde and copper sulphate methods for the control of grain smuts and certain other diseases. According to the several German publications on the subject, these mercury compounds cause no seed injury, but produce a stimulative effect. Copper carbonate was first used in Australia by Darnell-Smith (3), who obtained good results in the control of bunt of wheat. Satisfactory results have been obtained in the control of bunt of wheat in this country by Mackie and Briggs (10) and Heald and Smith (5). Only two varieties, Han River and Tennessee Winter, were treated with these compounds. Heavily smutted seed was used for these experiments, as also for the hot-water and formaldehyde treatments. All the seed used in the fall of 1921 was smutted artificially with covered smut, in addition to having been taken from seed lots showing heavy natural infestation.

Copper carbonate (C.P. basic) dust was shaken with the grain in a closed jar until there was a thin film of the dust over the surface of each kernel. Enough carbonate was added so that a small residue remained in the container, thus insuring a thorough application.

Chlorophol was used as a solution. The grain was soaked one hour in a 0.3 per cent solution and spread to dry before sowing. This strength was found to be effective in the control of oat smuts in preliminary experiments.

The results with these new treatments are so striking it seemed well to include them, even though they represent only one year of experiment. The results with copper carbonate indicate that this dust, though not injurious to the seed, will not satisfactorily control barley smuts under these conditions. The results with chlorophol, on the other hand, confirm the results obtained in Germany by the use of closely related organic mercury compounds, in that it causes no seed injury and, at the same time, gives an excellent smut control, averaging better than any other treatment, while the yields are very good. Tables 2 and 3 show the results of these experiments in comparison with those from the hot-water and formaldehyde treatments, and with untreated seed. Percentages of field germination given in these tables were obtained by

sowing 400 kernels from each treatment in the field on October 12, and counting the resulting plants on November 22. The percentage of field stands were estimated the following spring on the basis of the amount of space occupied by plants.

TABLE 2—*Results showing comparative value of seed treatments for the control of loose and covered smuts of Han River and Tennessee Winter barleys at Arlington Farm, Va., in 1922.*

Pl. no.	Variety	Treatment	Percentage of field germination	Percentage of field stand in spring	Percentage of smut		Yield bu. per acre
					Loose	Covered	
2	Han River (C.I. 2163)	Untreated	61.5	98	1.0	11.1	55.8
3		Copper carbonate	72.2	95	2.2	6.4	54.6
4		Chlorophol	68.0	98	0.6	1.3	61.2
5		Formaldehyde	65.0	85	trace	5.16	47.7
6		Hot water	62.0	95	0.2	2.5	57.2
24		Untreated	61.5	75	0.15	2.7	38.0
25	Tennessee Winter (C.I. 257)	Copper carbonate	72.2	92	0.85	2.77	35.7
26		Chlorophol	68.0	95	trace	0.2	47.5
27		Formaldehyde	65.0	75	0.28	6.7	39.2
28		Hot water	62.0	65	trace	0.35	38.4
7		Hot water	77.2	95	0.6	2.5	48.1
8		Untreated	78.7	100	1.6	4.7	48.5
9		Formaldehyde	75.2	95	0.2	2.4	50.4
10		Chlorophol	80.7	100	trace	0.1	58.7
11		Copper carbonate	82.2	98	0.9	1.9	53.3
12		Hot water	77.2	98	0.0	0.1	60.4
29		Untreated	78.7	98	0.5	1.85	46.7
30		Hot water	77.2	95	0.0	0.38	46.2
31		Formaldehyde	75.2	98	trace	2.5	54.6
32		Chlorophol	80.7	99	trace	0.2	56.4
33		Copper carbonate	82.2	98	3.67	5.26	50.4
34		Hot water	77.2	92	0.2	3.2	40.0

The results given in tables 2 and 3 show that in these experiments chlorophol was the most satisfactory treatment used. Field germinations were better with copper carbonate; but field stands in the spring, estimated on the basis of space occupied by plants, showed chlorophol to be superior in each case. This advantage is effectively shown in the yields. Even though the percentages of loose smut are very low, the results show that chlorophol was about as effective in controlling this smut as was formaldehyde or hot water and much more effective in the

TABLE 3—Average results from 2 plats showing effects of seed treatments for the control of smuts in Han River and Tennessee Winter barleys at Arlington Farm, Va., in 1922

Variety	Treatment	Average field germination	Average field stand—spring	Average percentage smut*		Average yield in bu.
				Loose	Covered	
Han River (C.I. 2163)	Untreated	61.5	86.5	0.6	6.9	46.9
"	Copper carbonate	72.2	93.5	1.5	4.58	45.1
"	Chlorophol	68.0	96.5	0.3	0.75	54.3
"	Formaldehyde	65.0	80.0	0.14	5.9	43.4
"	Hot water	62.0	80.0	0.1	1.4	47.8
Tennessee Winter	Untreated	78.7	99.0	1.05	3.27	47.6
"	Copper carbonate	82.2	98.0	2.28	3.58	51.8
"	Chlorophol	80.7	99.5	trace	0.15	57.6
"	Formaldehyde	75.2	96.5	0.1	2.45	52.5
"	Hot water ¹	77.2	95.0	0.2	1.54	48.9

¹ Average of 3 plats.

control of covered smut. The cause for the percentages of loose smut being higher in the copper carbonate plats than in plats from untreated seed is unexplained unless the increased stimulation caused the weak seeds, which might have been infected and otherwise would not have germinated, to germinate and produce smutty plants. The treatment may have influenced the development of the loose smut in some other way. The percentages are so low that the differences may be due to experimental error.

CONCLUSIONS

(1) Formaldehyde, which has been recommended for the control of covered smut of barley, controlled loose smut better than it controlled covered smut.

(2) Hot water controlled loose smut about as well as did formaldehyde and controlled covered smut slightly better.

(3) On an average, both the hot water and formaldehyde treatments caused seed injury and little or nothing was gained by using them.

(4) There is a varietal difference in the behavior of barleys toward seed treatment. These treatments might be recommended for the control of smuts in Tennessee Winter, while they could not be recommended for such varieties as Greece and Wisconsin Winter.

(5) The efficiency of these treatments may vary under different local conditions. Furthermore, other varieties might behave differently toward them.

(6) Where the percentages of smut are not high, the results would not justify the use of the modified hot-water and formaldehyde treatments employed in these experiments.

(7) Of the two treatments, formaldehyde is much more easily applied, and, according to these results, is as efficient as hot water.

(8) In experiments of a single year, copper carbonate dust failed to control the smuts of barley, and apparently caused an increase in the percentages of loose smut. However, it had a favorable effect on seed germination.

(9) Chlorophol reduced the amount of loose smut, controlled covered smut effectively and caused an increase in field germination, field stands and yields of Han River and Tennessee Winter barleys in a single year of experiment.

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AN IMPROVED FORMALDEHYDE TANK FOR THE ONION DRILL

P. J. ANDERSON AND A. V. OSMUN

WITH THREE FIGURES IN THE TEXT

For controlling onion smut the only successful method which has been discovered is the application of formaldehyde to the seed and soil in the furrow at planting time. Selby¹ in 1902, was the first to apply this by means of a tank attached to the seeder. His apparatus consisted of an old maple syrup can with a tin outlet tube and "a suitable shut-off arrangement" which was suggested but not described. Stone², in 1909, described a more improved copper or galvanized iron tank which was attached in front of the seed box and partly over the wheel of the Planet Junior seeder. The outlet pipe was a quarter inch block tin flexible pipe in the course of which was inserted a globe valve for regulating the flow of formaldehyde solution. In 1914, Stone³ described two other kinds of tanks, one which surrounds the seed box like a horseshoe and the other a somewhat cubical tank adjusted between the handles just back of the seed box. Each of these had a capacity of two gallons while his first tank held only one gallon. In the later models "an improvement over the earlier apparatus consisted in an iron rod attached to the valve and leading to the handles of the sower by means of which the operator could adjust the flow at will." Larger tanks which are attached to two-row drills and to eight-row horse drills were figured by Fernald and Osmun⁴ in 1916. Various other modifications in the shape, position and capacity of the tanks have been made by plumbers and practical growers in the Connecticut Valley and are in use here but

¹ Selby, A. D. The prevention of onion smut. Ohio Agric. Exp. Sta. Bull. 131: 50. 1902.

² Stone, G. E. The control of onion smut. Massachusetts Agric. Exp. Sta. Circ. 21. 2 p., 2 fig. 1909.

³ Stone, G. E. The control of onion smut. Massachusetts Agric. Exp. Sta. Circ. 41. 4 p., 4 fig. 1914.

⁴ Fernald, H. T. and A. V. Osmun. The control of onion diseases and thrips. Massachusetts Agric. Coll. Extens. Circ. 5. 4 p., 5 fig. 1916.

in principle they are all the same. Similar tanks have been used by pathologists in Wisconsin,¹ New York, Oregon² and other states.

Renewed investigation on the control of smut was begun by the Department of Botany of the Massachusetts Agricultural Experiment Station in 1918. It was found at the very outset that no onion grower in the Connecticut Valley could be induced to use the extreme dilutions commonly recommended by experiment stations and push across the field the weight of water which such dilutions required, viz., about 200 gallons to the acre. Disregarding the experiment station recommendations, they were using more concentrated solutions and applying a smaller quantity per unit of row. The results were sometimes successful in controlling smut, sometimes worthless, at other times disastrous because they killed the seed with too great a concentration. Early in the investigation it became apparent that so much water was not necessary during ordinary seasons on the onion fields of the Connecticut Valley. The investigation finally resolved itself into an attempt to answer this question: What is the maximum concentration of formaldehyde and the minimum quantity of the same solution which will give profitable control of smut? Incidentally it should be mentioned that the onion grower does not object to a certain amount of injury to the seed since he can counterbalance this loss by setting the drill for a little more seed. A few extra pounds of seed is less expensive than the extra labor required to apply a safe dilute solution in larger quantities. The plan of the work was to try out in the smutted fields different concentrations of the formaldehyde and different quantities per unit of row and then keep accurate data on the amount of injury to the seed and on the control of smut. By continuing these tests through a series of years it was hoped that the data collected would answer the question stated above.

In order that the data obtained might be reliable, a machine was needed which would distribute evenly and with a fairly high degree of accuracy any desired quantity of solution on a stated length of row. For this purpose the regulating apparatus on all of the machines which are mentioned above was found to be worthless. None of them had a valve which could be set so that the operator would know when he started across the field how much solution would be applied per hundred feet. Actual tests of the different types of tanks showed considerable variation in the flow depending on how full the tank was. A 14-quart tank de-

¹ Walker, J. C. Onion diseases and their control. United States Dept. Agric. Farmers' Bull. 1060. 23 p., 12 fig. 1919.

² Barss, H. P. Onion smut control. Oregon Agric. Exp. Sta. Crop Pest and Hortie. Rept. 3 (1915-20): 187. 1921.

scribed below required more than twice as long to deliver the last quart as it did the first. The one-gallon copper tank first constructed by Stone—which is still in the possession of the Station—was tested and showed a difference in time of 15% between the first and last pint. None of the tanks previously described have any method of correcting this error. None of them have any provision for eliminating the error due to variation in the speed of the operator. In short, there were so many sources of error and variation in all of the machines described up to the present that they were found to be utterly unsuitable for accurate

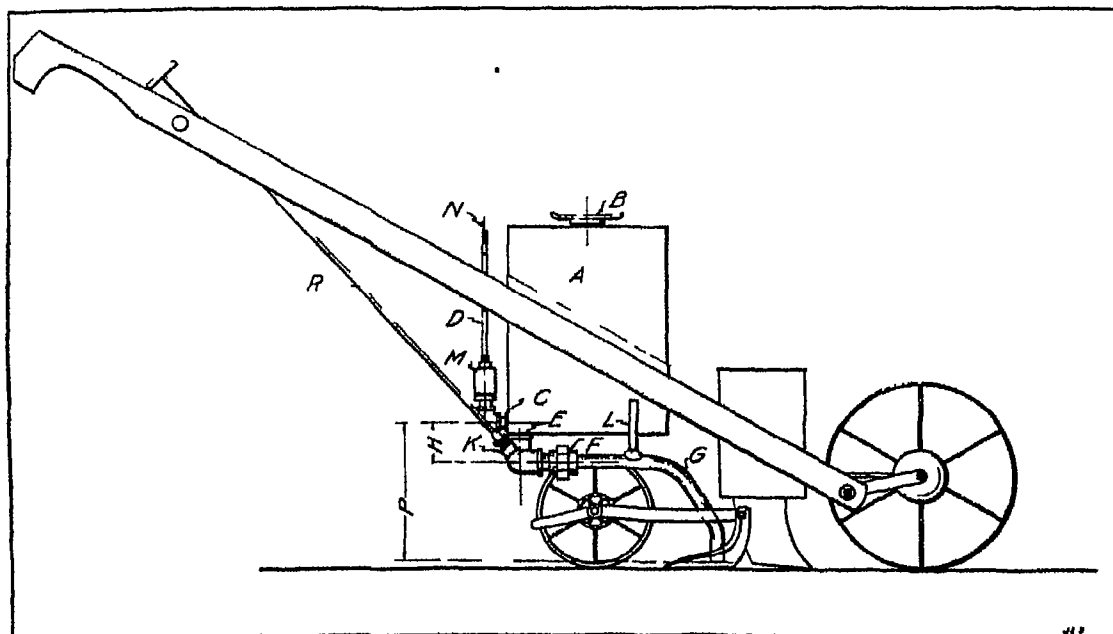


FIG. 1. Diagram of the tank, attached to Planet Junior seeder.

research studies and a new tank and method of regulation had to be devised.

Attempts were first made to calibrate the valve in the outlet pipe and, by means of a semicircular dial (on the cross bar of the handles of the planter) with numbered notches in the edge, to set the opening in the valve to permit the flow at any desired speed. Knowing the amount of solution which he wished to apply to a unit of row, it was first necessary for the operator to measure the speed at which he walked and then set the control lever at the proper notch on the dial. This arrangement was not quite satisfactory because it was necessary that the valve be completely closed in $\frac{1}{2}$ revolution of the spindle and no valve could be found which would do this with even approximately equal increments. There was also sufficient "play" or wear in the parts of the valve or the

control lever to introduce variations in the flow even when the lever was set at the same notch on the dial at successive times. A gate valve and other types of valves were tried but all were found to be more or less unsatisfactory. Finally, the valve was abandoned as a means of regulating the flow and was used in our improved tank only for turning the stream on or off. The final improved form of the apparatus, which has

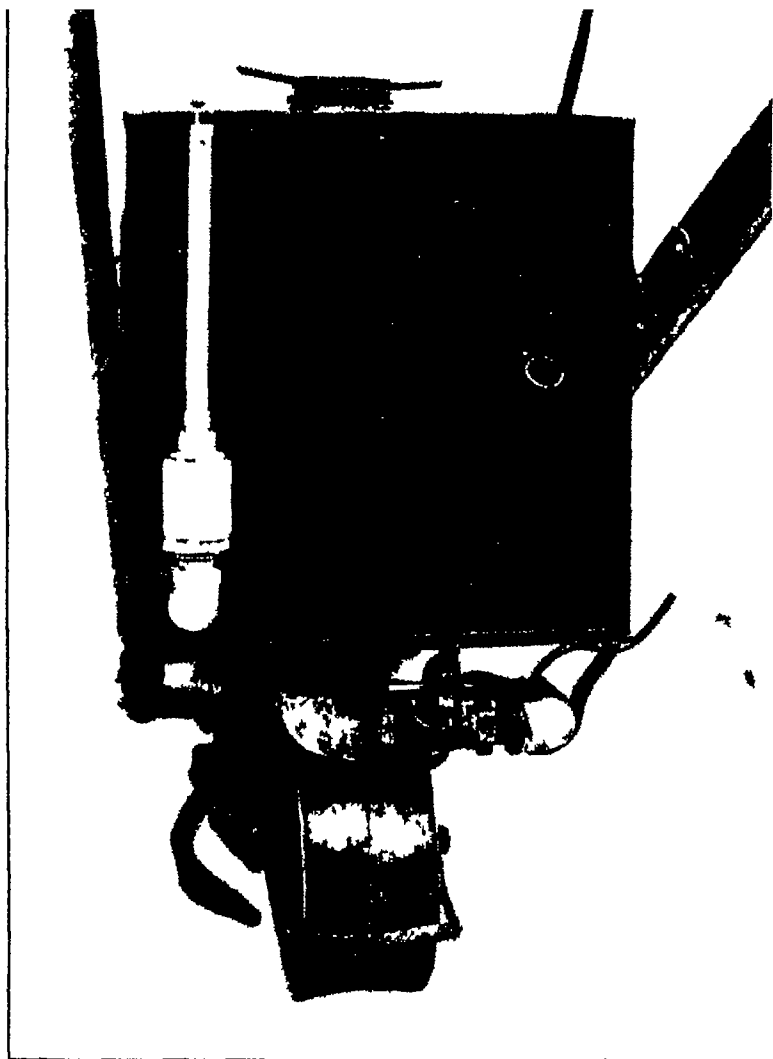


FIG. 2. Rear view of tank showing stand-pipe and arrangement of outlet pipe.

given satisfactory results in our field experiments, is described below and illustrated in the diagrammatic text figure 1.¹

The tank itself (A) is a 14-quart cubical galvanized iron box attached between the handles of the drill just back of the seed box. The bottom

¹ This apparatus was completed in cooperation with the Department of Rural Engineering and credit for the working out of the mechanical details should be given to Professors C. I. Gunness and J. L. Strahan of that department.

of the tank is not flat but has a slight slope to a lowest point at the rear from which the solution is conducted through a $\frac{1}{2}$ -inch pipe (E) to the stopcock (K) and to the union (F). From there it is led through a $\frac{3}{8}$ inch flexible block tin tube (G) and distributed on the seed and soil just back of the seed spout and in front of the coverers. The valve (K) is operated by a $\frac{1}{4}$ inch iron rod (R) from the rear end of the handles. It has nothing to do with the regulation of the flow but merely starts or stops the stream at the ends of the row or wherever desired. So much of the apparatus is not essentially different from that used by the previous investigators mentioned above. The modifications now to be described, however, have, so far as the writers know, never been used by other investigators.

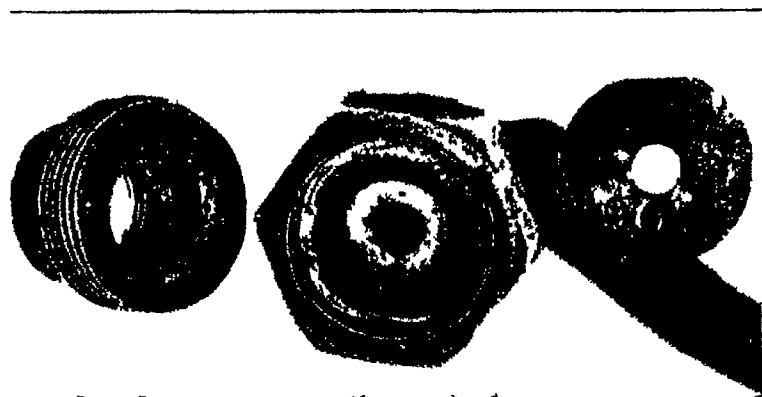


FIG. 3. Union in the outlet pipe taken apart to show position of disk and washers. Also a stamped disk at the left.

The rate of flow from the tank is regulated by a series of brass disks with central apertures of graded sizes. The disk is held in place between rubber washers in the union (F) of the outlet pipe (Fig. 3). Any number of these simple brass disks may be quickly cut out with tin shears from a sheet of brass. A hole is drilled through the center of each disk and this is enlarged with a rat-tail file to the proper size for the delivery of a gallon of solution in the previously calculated number of seconds which the operator wishes to use. The number of seconds required to deliver a gallon of solution is then stamped on the disk (Fig. 3). The size of the aperture needed depends on the formula of application which the operator wishes to use and the rate at which he walks. The number of disks which the experimenter or grower needs depends on the number of formulas he wishes to use and the number of men, of different speeds of walking, who expect to push his drill. Thus in our own experimental work seven different formulas of application were being tested and the drill was pushed at different times by three

operators, the first one walking 4 ft. per second, the second one $4\frac{1}{2}$ ft. per second and the third one 5 ft. per second. Thus before the experiments were completed we had 21 disks. The operator could quickly find the proper disk by reference to a table attached to the side of the tank (Table 1):

TABLE 1: *Indicating the disks of the onion drill to be used according to the speed of workman and formula desired.*

Formula	One gallon of the solution on	4 ft. per sec.	$4\frac{1}{2}$ ft. per sec.	5 ft. per sec.
1- 50-3000 ¹	480 ft.	120	106	96
1- 50-4000	640	160	142	128
1- 50-5000	800	200	178	160
1- 32-3000	750	188	167	150
1- 32-4000	1000	250	222	200
1- 64-3000	375	94	83	75
1-128-3000	187.5	47	42	37.5

To illustrate: if the operator found that he walked at the rate of $4\frac{1}{2}$ ft. per second and he wished to use the 1-64-3000 formula, a glance at the table would tell him to insert in the union the disk stamped with the figure 83. Such a large number of disks will be needed only by the experimenter. The practical grower would not have occasion to use more than two or three and could make them as desired. This method of regulation was found to be very satisfactory and has the advantages of (1) permanent accuracy; no variation with wear or play; (2) cheapness and ease of construction within the reach of any grower; (3) simplicity; no complicated mechanism to be frequently out of repair, and (4) great elasticity, since a disk may be quickly made to suit any desired rate of flow.

The next modification was made for the purpose of correcting the variation in flow due to difference in head of the liquid as the quantity in the tank diminishes. All of the tanks which the writers have seen described have loose-fitting lids in the top through which the solution is poured to fill the tank. As the solution flows out through the outlet pipe the space which it leaves in the tank is immediately filled with air which comes in around the lid. Such an arrangement makes the rate of flow entirely dependent on the height of the water in the tank and as a result, the flow is much more rapid when the tank is full than when it is nearly empty. In order to determine the extent of such variation, the

¹ This formula means that 1 pint of commercial formaldehyde was diluted in 50 pints of water and applied to 3000 feet of onion row.

14-quart tank just described was filled and the water permitted to run out with the lid loose or removed entirely. The time required for delivering each of the successive quarts was determined with a stop watch and the average of 3 tests is indicated in table 2:

TABLE 2: *Rate of flow of each successive quart of liquid from 14-qt. tank (open).*

Quart	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Seconds	17.7	18.8	19.2	20.0	20.5	21.6	22.8	24.3	25.2	26.9	29.3	32.3	34.6	39.4

Thus the operator who started with a full tank would be putting on the first part of the treated row double the amount which he puts on the last part. The resulting control or injury would naturally show a similar variation. Data derived from the use of such a tank cannot be accurate. The variation in even the small tank described by Stone has been noted above. In order to make our control data reliable it was absolutely essential that this variation be eliminated. After considerable experimentation this was finally very successfully accomplished by modifying the tank thus: Instead of a loose-fitting lid, a screw cap (B), of 2-inch diameter (such as is commonly used on an automobile radiator) is used for closing the opening in the top. When screwed down with the shoulder against a rubber washer, this makes the top air-tight. A stand-pipe (D) parallel with and as high as the side of the tank is connected with the air vent (C) by means of an elbow. In this way, atmospheric pressure is maintained at the level of the vent (C) and consequently a uniform flow of solution is obtained irrespective of the height of water in the tank. The effective head producing the flow is always equal to (H). In order to prove that the flow is uniform, the outflow of each successive quart was timed just as described above but with the air-tight cap screwed down, and (with the no. 150 disk in the union) every quart from the first to the 14th flowed out in just $37\frac{1}{2}$ seconds.

Two other modifications of minor importance have been added. In the enlarged base of the stand-pipe there is a float (M), which consists of a light hollow brass cylinder which has a "play" up and down of about 1 inch. Attached to the top of this float is a slender brass wire stem (N) passing up through the narrow part of the stand-pipe and projecting an inch above the top of it when the float is up but just even with the top when the float is down. When the stream is running from the tank and everything is in order, the float is down and the stem does not appear above the top. If, however, there is any leak in the top of the

tank or the operator has forgotten to screw down the cap, i. e. if the tank for any reason is not air-tight, the water rises in the standpipe to the level in the tank. the float remains up and the projecting stem warns the operator that something is wrong. The float is thus a safety device and not an absolutely essential part.

The second minor modification is a very small pipe (L) about 2 inches long inserted on the upper side of the outlet pipe. This was added when it was discovered that for each of certain intermediate disks there were two distinct rates of flow. If the pipe below the union became immediately filled with water when the stream was turned on, it continued to come out in a steady stream and we had the faster rate of flow. Under these conditions the effective head would be (P) in the diagram. But at other times, even with the same disk, air gained access to this pipe and the water dribbled out. Then we had the slow rate of flow due to the head (H). Either flow remained constant but one never knew when he opened the valve which speed he would have. This difference in flow was eliminated when air was admitted to the outlet pipe through the small opening and pipe just mentioned.

With this improved tank just described all chances of error due to variation are eliminated except one. Variation in the rate of application might arise from variation in the speed at which the workman pushes the drill; e. g., he might walk more slowly when he was tired or in softer land, etc. Numerous tests of the speed of different workmen at various times have convinced the writers, however, that a man's speed is remarkably constant and the error from this source is small. Nevertheless, it would be a distinct improvement if even this small error could be eliminated and the practical grower would be saved the trouble of measuring his speed and calculating the disk which he should use. This could be done if the apparatus were so constructed that the amount of solution delivered was proportionate to the distance travelled (i. e., to the number of revolutions of the drill wheel) and not dependent on the length of time during which the valve is open. Then the rate of application would be entirely independent of the speed at which the drill was pushed. Such a regulating apparatus, geared to the drill wheel, is mechanically possible and its construction has been planned at various times by the writers. The construction has been abandoned for various reasons but principally because it would be too complicated and expensive and therefore less suitable for the average onion grower. For all practical purposes, and even for a fairly high degree of accuracy for experimental work, the apparatus described above is quite satisfactory.

THE DUSTING OF WHEAT AND OATS FOR SMUT

F. D. HEALD, G. L. ZUNDEL AND L. W. BOYLE

WITH ONE FIGURE IN THE TEXT

INTRODUCTION

The control of bunt or stinking smut (*Tilletia tritici*) is the important problem in the wheat-growing section of eastern Washington. The treatment of seed wheat with bluestone or copper sulphate has predominated in the Palouse region, while in the wheat-growing regions of central Washington formaldehyde has been the fungicide usually employed.

There are, however, objections to the use of either copper sulphate solution or to formaldehyde in that both fungicides impair the germinating power (8, p. 17 and 13, p. 25-30) of the wheat so treated. When formaldehyde was first introduced, about 1897, it was thought that it would not injure the germinating quality of the treated wheat. This was soon proven to be an erroneous assumption.

It has been known for a long time that much of the injury from treating wheat in a solution of copper sulphate could be overcome by an after bath of milk of lime. Recently (14) it has been shown that the milk of lime is also effective in counteracting the ill effects of formaldehyde.

HISTORICAL

From work done in 1915 and 1916, Darnell-Smith (2) in 1917 reported excellent control of bunt by the use of copper carbonate dust. In addition to excellent control of bunt he showed that there was no injury to the seed. In 1919, Darnell-Smith and Ross (4) reported again on the use of copper carbonate to control bunt. In the latter part of 1919 Perkins (12) criticized the work of Darnell-Smith and Ross and rather questioned the efficacy of copper carbonate as a fungicide. In 1921 Darnell-Smith (3) answered the objections of Perkins to the use of copper carbonate. The work of the Australian investigators attracted the attention of American investigators and in 1920 and 1921, Mackie and Briggs (10, 11) of California published data confirming the work done by Darnell-Smith and Ross. In 1922, Lambert and Bailey (9) again confirmed the Australian investigations and further reported that oat smuts could be controlled by applying copper carbonate dust

to the seed. Again in 1922, Heald and Smith (6, 7) in Washington and Coons (1) in Michigan report good control of bunt by the use of copper carbonate, while the former authors have designed a special dusting machine which has been used with good results.

FIELD DEMONSTRATIONAL WORK

Bunt Control.—In the spring of 1921, field demonstrations using copper carbonate dust for bunt control were carried out in Franklin county in co-operation with the county agent. The results showed the superiority of copper carbonate over formaldehyde.

In the fall of 1921 in cooperation with the county agents the demonstrational work was extended to nine of the principal wheat-growing counties of the state, viz. Adams, Benton, Columbia, Franklin, Kittitas, Pend d'Oreille, Stevens, Walla Walla and Whitman. Over ten thousand acres of wheat were seeded in which the seed had been treated with copper carbonate, using two ounces of the powder for each bushel of wheat. This seed was planted in plots of varying size to compare it with the farmer's own treatment in which bluestone or formaldehyde was used.

It was observed that seed wheat treated with copper carbonate germinated from one to five days sooner, and the plants were more vigorous. There was very little seed injury to the copper carbonate treated seed. In a number of cases where seed was treated with formaldehyde or bluestone, there was severe winter killing, while seed planted in the same field and treated with copper carbonate overwintered uninjured.

Just before harvest (1922) each field demonstration was visited and the amount of smut was determined in the copper carbonate plots and in the parts of the field in which bluestone or formaldehyde was the fungicide used. Ten counts of 100 heads of wheat each were made for each treatment in order to determine the percentage of smut.

The appended table (Table 1) gives the average percentage of smut for the different treatments in the various counties. In case no smut was found in the field, it was not included in the average. Eight trial fields in Adams County, four in Franklin, one in Columbia and two in Kittitas were free from smut.

Oat Smut Control.—Lambert and Bailey (9) first reported the use of copper carbonate to control the smuts of oats. In the spring of 1922, five field demonstrations were started in Skagit County, in cooperation with the county agent to test the comparative value of formaldehyde and copper carbonate. Each bushel of oats was treated with two ounces of copper carbonate. The standard formaldehyde treatment was used.

Just before harvest, the county agent, C. H. Bergstrom, determined the percentage of smut by making ten counts of 100 panicles each for each treatment. The average percentage of smut in the five demonstrations was as follows:

Untreated 8.9; formaldehyde 0.20; formaldehyde + lime after bath 1.10; copper carbonate 1.17.

TABLE 1—Summary of farm demonstrations showing comparative effectiveness of blue-stone, formaldehyde and copper carbonate in the control of bunt 1922

County	No. of co-op-erators	Average percentage of smut							
		Blue-stone	Copper carbonate	Blue-stone Lime	Copper carbonate	Formaldehyde	Copper carbonate	Formaldehyde + Lime	Copper carbonate
Adams	3	10.4	8						
Franklin	3					4.2	.4		
Benton	2	8	.45						
Columbia	8	12.9	8.5	9.7	8.1	18.1	11.	19.6	6.6
Pend Oreille	1					35.	3.7		
Stevens	5					4.9	2.		
Kittitas	1	2.2	.2	0.3	.2	1.6	.2	0.4	2
Whitman	13	16.5	10.8	14.6	10.8	12.9	6.7		
Walla Walla	12	9.4	9.1			16.0	9.		
Wash. State Avg.		10.9	7.2	11.0	8.5	9.1	3.1	10.2	3.4

EXPERIMENTAL WORK

A series of experiments relative to the use of copper carbonate dust treatment for control of cereal smuts have been carried out during the past two seasons. These experiments were outlined to determine the following points: (1) The comparative protection of various fungicides for stinking smut of wheat from seed-borne spores; (2) the effect of certain seed treatments on prevention of bunt from soil contamination (summer fallow); (3) to what extent, if any, copper carbonate dust treatment for smut injures the seed and if extended storage before planting makes a difference; (4) to what extent copper carbonate dust is effective in the control of oat smut (*Ustilago levis* (K. & S.) Mag.).

THE COMPARATIVE TESTS OF VARIOUS FUNGICIDES FOR BUNT CONTROL IN SPRING WHEAT

In making these comparative tests, three varieties of seed were used, namely, Marquis, a resistant "vulgate" type, Bluestem, a susceptible

"vulgare" type, and Jenkins Club, a susceptible "compactum" type. Before treatment all the seed was artificially smutted by shaking or churning it in a flask with one of three different amounts of smut powder. (Smut powder is prepared by grinding smutted heads through a food chopper and sifting through a fine sieve.) The amounts of smut powder used and approximate number of smut spores carried per grain of wheat were as follows:

Light	smutting—0	1 gr. smut powder per 100 gr. ms. seed = 9,000 spores per grain
Medium	" —0 4	" " " " " " " " " " 36,000 " " "
Heavy	" —1.0	" " " " " " " " " " 90,000 " " "

Heavily smutted seed appeared black and carried a much greater number of spores than seed used in ordinary farm practice. The dust treatments were applied in the same way, by shaking the smutted seed in a large flask with the powder. The seed was hand planted in duplicate rod rows, care being taken after planting a heavily smutted sample, to wash the hands before planting a sample of lighter smutting. At harvest counts were made to determine percentage of clean plants, percentage of partially smutted plants, percentage of totally smutted plants and percentage of smutted heads.

The first series of plantings were made of seed treated with varying amounts ($\frac{1}{2}$ to 3 oz. per bu.) of copper carbonate dust to determine if possible the minimum amount which could be used to obtain a practical maximum protection. Besides the treatments with pure copper carbonate, hydrated lime was used as a diluent with equal amounts of copper carbonate.

The maximum percentage of smutted heads produced in any planting of mediocre smutting, even with reduced amounts of copper carbonate, was 0.9, the average being 0.21 per cent as compared with 24.26 per cent smutted heads in control plantings of untreated medium smutted seed. The average percentage of smutted heads produced by heavily smutted treated seed was 0.97 as compared with 40.5 per cent produced by controls of heavily smutted untreated seed. The average for all copper carbonate treatments was 0.41 per cent. Copper carbonate treatments of spring wheat, using two ounces per bushel, carried out in 1921 gave complete protection from bunt, as reported by Heald and Smith (6), but in our 1922 tests it required 3 oz. per bushel to give a smut-free crop.

The second series of plantings was made with seed smutted in the same way but treated with various amounts of finely powdered anhydrous copper sulphate mixed with equal amounts of either finely powdered calcium carbonate or hydrated lime. Such mixtures should produce copper carbonate in contact with soil moisture and were treated with the

TABLE 2—*Results in control of stinking smut, using various quantities of copper carbonate dust. Spring seeding 1922.*

Treatment	Variety	Average percentage of smutted heads for three degrees of smutting	Degree of smutting	Average percentage of smutted heads for three varieties
Copper carbonate ½ oz. per bushel	Marquis	0	Light	.29
	Bluestem	0	Medium	0
	Jenkins Club	1.65	Heavy	1.03
Copper carbonate ½ oz. plus hydrated lime ½ oz. = 1 oz. per bushel	Marquis	0	Light	0
	Bluestem	.42	Medium	.27
	Jenkins Club	2.01	Heavy	2.16
Copper carbonate 1 oz. plus hydrated lime 1 oz. = 2 oz. per bushel	Marquis	0	Light	.12
	Bluestem	1.90	Medium	.44
	Jenkins Club	.38	Heavy	1.72
Copper carbonate 1 oz. per bushel	Marquis	0	Light	0
	Bluestem	.26	Medium	.26
	Jenkins Club	0	Heavy	0
Copper carbonate 2 oz. per bushel	Marquis	0	Light	0
	Bluestem	.29	Medium	.3
	Jenkins Club	.6	Heavy	.59
Copper carbonate 3 oz. per bushel	Marquis	0	Light	0
	Bluestem	0	Medium	0
	Jenkins Club	0	Heavy	0

idea of substituting the cheaper mixture for the more expensive copper carbonate. The plantings of seed treated with anhydrous copper sulphate mixed with hydrated lime gave an average of 1.65 per cent smutted heads as contrasted with an average of 23.3 per cent in all untreated controls. The anhydrous copper sulphate mixed with calcium carbonate averaged 0.77 per cent, which gives it a preference over the hydrated lime mixture. The average for all the anhydrous copper sulphate mixtures was 1.4 per cent smutted heads as contrasted with 0.41 per cent smutted heads for all the copper carbonate tests. No complete protection was afforded except in the case of the highly resistant variety, Marquis.

TABLE 3—*Results in control of bunt, using various amounts of anhydrous copper sulphate.*¹
Spring seeding 1922.

Treatment	Variety	Average percentage of smutted heads for three degrees of smutting	Degree of smutting	Average percentage of smutted heads for three varieties
Anhydrous copper sulphate $\frac{1}{2}$ oz. plus calcium carbonate $\frac{1}{2}$ oz. = 1 oz. per bushel	Marquis	0	Light	.10
	Bluestem	1.74	Medium	1.8
	Jenkins Club	1.96	Heavy	1.8
Anhydrous copper sulphate 1 oz. plus calcium carbonate 1 oz. = 2 oz. per bushel	Marquis	0	Light	.12
	Bluestem	.34	Medium	.9
	Jenkins Club	.51	Heavy	.63
Anhydrous copper sulphate 2 oz. plus calcium carbonate 2 oz. = 4 oz. per bushel	Marquis	0	Light	.42
	Bluestem	1.56	Medium	.32
	Jenkins Club	.81	Heavy	1.61
Anhydrous copper sulphate $\frac{1}{2}$ oz. plus hydrated lime $\frac{1}{2}$ oz. = 1 oz. per bushel	Marquis	.37	Light	.90
	Bluestem	4.93	Medium	1.68
	Jenkins Club	.84	Heavy	3.55
Anhydrous copper sulphate 1 oz. plus hydrated lime 1 oz. = 2 oz. per bushel	Marquis	0	Light	0
	Bluestem	1.25	Medium	.275
	Jenkins Club	.87	Heavy	1.84
Anhydrous copper sulphate 2 oz. plus hydrated lime 2 oz. = 4 oz. per bushel	Marquis	0	Light	.29
	Bluestem	.54	Medium	.84
	Jenkins Club	1.14	Heavy	.54

The third series of plantings was made with seed smutted in the same way as the others, but treated with the Corona² compound of copper carbonate for comparison with pure copper carbonate (Table 4). The Corona compound contained 17 to 18 per cent metallic copper as compared with about 50 per cent in the pure copper carbonate. The

¹ See table 4 for controls.

² Furnished by the Corona Chemical Division of the Pittsburgh Plate Glass Company, Milwaukee, Wisconsin.

average percentage of smutted heads in plantings of comparative samples were as follows:

$\frac{1}{2}$ oz. copper carbonate per bushel,	0 54	per cent smutted heads	
1 " " " " "	0 087	" " "	
2 " " " " "	0 29	" " "	
3 " " " " "	0 00	" " "	
2 " Corona compound " "	0 077	" " "	
4 " " " " "	0 00	" " "	
No treatment No. 1	17.88	" " "	
No treatment No. 2	28.72	" " "	

This single test, however, indicates that Corona compound has a better protective action than pure copper carbonate carrying an equivalent of metallic copper. With this series were made plantings of seed treated with Seed-O-San, an organic mercury compound furnished by Standard Seed Tester Company, Chicago, Illinois. This is also in dust form and applied in the same way. It did not give as good protection as the copper dusts in plantings of heavily smutted seed, but was quite effective in the plantings of light and medium smutted seed. (See table 4.)

COMPARATIVE TESTS OF VARIOUS FUNGICIDES FOR BUNT CONTROL IN WINTER WHEAT

When planting wheat in the fall, the problem has been how to control reinfection of seed from wind blown smut spores in the soil. Previous experiments have shown nearly perfect control of smut by the use of bluestone, formaldehyde and copper carbonate treatments when this was not a factor. A series of comparative tests were made to determine the effectiveness of various fungicides in control of smut from soil contamination. Three varieties were used: Hybrid 128, a very susceptible "compactum" type; Turkey Red, a very resistant variety; and Red Russian, a rather susceptible variety. These were not artificially smutted as in experiments with spring wheat, but were planted in duplicate row rows on summer fallow which had been artificially smutted. The smutting was done with a hand duster to insure as uniform distribution of the powdered smut as possible. The soil then contained far more smut than normal summer fallow.

Assuming no protective action from the formaldehyde treatments (average 25 per cent smutted heads) all of the copper treatments showed a reduction (15.12 per cent smutted heads) in resulting amount of smut. Copper carbonate dust treatment averaged 18 per cent smutted heads, which is in excess of the average amount in the several tests with bluestone. It is significant that the copper sulphate sprinkle, using 1 pound

TABLE 4—*Results in control of stinking smut, using Corona compound of copper carbonate, Seed-O-San, and controls of untreated smutted seed. Spring seeding 1922*

Treatment	Variety	Average percentage of heads of three degrees of smutting	Degree of smutting	Average percentage of smutted heads for three varieties
Corona compound of copper carbonate 2 oz. per bushel	Marquis	0	Light	0
	Bluestem	.08	Medium	0
	Jenkins Club	.15	Heavy	.23
Corona compound of copper carbonate 4 oz. per bushel	Marquis	0	Light	0
	Bluestem	0	Medium	0
	Jenkins Club	0	Heavy	0
Seed-O-San 2 oz. per bushel	Marquis	0	Light	.18
	Bluestem	1.48	Medium	.18
	Jenkins Club	.60	Heavy	1.72
Control No. 1 untreated smutted seed	Marquis	.43	Light	3.63
	Bluestem	25.8	Medium	12.58
	Jenkins Club	27.63	Heavy	39.5
Control No. 2, untreated smutted seed	Marquis	.71	Light	6.52
	Bluestem	41.85	Medium	37.99
	Jenkins Club	43.58	Heavy	41.64

to 1 gallon of water at the rate of $\frac{1}{2}$ gallon per bushel, gave the best protection, while sulphur showed but little protection.

A second series of plantings to determine the comparative value of various fungicides in control of smut from soil contamination was made in 1140 acre plots. Hybrid 128, Red Russian and Turkey Red were the varieties used in these plantings. Smut percentages were obtained by counting the total number of heads (1000-4000) in a drill row. It can be assumed that the smut (8.3 per cent) in the plantings of seed treated with formaldehyde 1 to 40 came from soil contamination and that the seed carried very little smut, since the average for the untreated seed was 8.64 per cent smutted heads (Fig. 1). All the copper treatment showed a reduction in the amount of smut over formaldehyde with the best protection afforded by the standard blue-stone treatment followed by the lime bath. The copper carbonate dust treatment gave a reduction of 2.78 per cent over formaldehyde as compared with 2.28 per cent reduction from anhydrous copper sulphate. The results obtained with the spring seedlings suggest that 3 oz. of copper carbonate per bushel

would have given an equal or better protection than the bluestone treatments.

TABLE 5—Comparative effect of seed treatments in preventing smut in winter wheat planted in artificially smutted summer fallow. Fall seeding 1921.

Treatment	Hybrid 128 Percentage smutted heads	Turkey Red Percentage smutted heads	Red Russian Percentage smutted heads	Average percentage smutted heads
Sulphur, 200 lbs. per A.	43 2	0 0	20 9	21.3
Sulphur, 20 lbs. per bu.	50 3	0 1	28 5	26.2
Formaldehyde 1-40 ...	49 6	5 2	23.8	26.2
Copper carbonate 2 oz. per bu.....	36 2	3 9	13 9	18 0
Anhydrous copper sul- phate 1 oz. calcium carbonate 1 oz.....	25.	6.2	21.3	18.5
Copper sulphate 1-5...	6.	4.3	27.5	12.6
Copper sulphate 1-5 plus lime bath....	21.3	2.9	22 3	15.5
Formaldehyde 1-40 ...	34 4	4.4	32.3	23.7
Copper sulphate 1-1 sprinkle.	15 9	1 5	15 7	11.03

THE EFFECT OF COPPER CARBONATE ON GERMINATION OF WHEAT

Besides being effective as a seed fungicide in control of stinking smut of wheat and oat smut, copper carbonate has no toxic effect upon treated seed (10, 11, 9, 6). This is of considerable advantage, since as stated before the commonly used formaldehyde and bluestone treatments injure the seed to a marked degree. Copper carbonate also has a stimulating effect, inducing more rapid germination and more vigorous growth.

Since there is often a congestion of work on the farm at the period when treating and sowing of seed is usually done, the question arises as to whether seed might be treated some months previous to seeding and

TABLE 6—*Comparative effects of seed treatments in preventing stinking smut in winter wheat planted in ordinary summer fallow. Fall seeding 1921¹*

Treatment	Hybrid 128	Turkey Red	Red Russian	Average percentage smutted heads for three varieties
	Percentage smutted heads	Percentage smutted heads	Percentage smutted heads	
Untreated	17.4	1 5	9.8	9 56
Copper carbonate 2 oz. per bushel	13 0	1 4	3 2	5 86
Anhydrous copper sulphate 1 oz. plus calcium carbonate 1 oz. = 2 oz. per bushel	14.8	0 5	3 8	6 36
Copper sulphate 1-5 . . .	10.5	0 6	3.8	4.96
Copper sulphate 1-5 plus lime bath	7.1	0 7	3.7	3.83
Formaldehyde 1-40	18.6	0 6	5.7	8.3
Untreated	14 7	1 4	7.1	7.73

stored without injurious effect. To determine this point, four common varieties of wheat were each treated with the standard amount (2 oz. per bushel) of copper carbonate dust. Comparative germination tests were made of this seed and untreated seed of the same lots. Monthly tests were made by planting 200 seeds of each sample in flats in the greenhouse and counting the number of seedlings. The results (See table 7) show that after a period of nine months no injury resulted, while the treated grain showed an average increased germination over untreated seed.

The effectiveness of copper carbonate in control of oat smuts.—Oat smut (*Ustilago levis*) like stinking smut of wheat is seed-borne and controlled by the same formaldehyde treatment. Seed injury and other disadvantages of this treatment, being overcome by use of copper carbonate

¹From an experiment in cooperation with Division of Farm Crops, Washington Agriculture Experiment Station.

dust in control of smut in wheat suggested possibilities of its effectiveness in control of smut.

In making this test the same methods of smutting, treating and planting the seed as described for wheat were used. Three varieties, Swedish Select and Alandine, both moderately susceptible and



FIG. 1. Turlay Red wheat fields on Nunamaker Ranch near McAdams, Franklin County, Washington. Fields seeded at rate of 70 pounds in November, 1921; photographed May 24, 1922. Light area from formaldehyde-treated seed; heavy stand from seed treated with copper carbonate. Photo by H. Simond.

Chinese Hulless, very susceptible, were used and the following three degrees of smutting were employed:

Light smutting	= 0.1 gr. of smut powder per 100 grams of seed
Medium smutting	= 0.4 " 100
Heavy smutting	= 1.0 " 100

The copper carbonate was used in amounts equal to 2, 3 and 4 oz. per bushel of seed.

The results (Table 8) shows the copper carbonate dust far more effective with the hulless variety than with the common varieties. The average

TABLE 7—*Comparative germination percent of untreated and dusted seed of several varieties of wheat*

Date of Test (1922)	Jenkins Club		Hybrid 128		Marquis		Jones Winter Fife	
	Un-treated	Cu CO ₃	Un-treated	Cu CO ₃	Un-treated	Cu CO ₃	Un-treated	Cu CO ₃
Feb. 7	85	88.5	87.5	92.5	89.5	94.5	95	90.5
Mar. 8	67.5	90	75	60	96	85	91	96
Apr. 15	81.5	—	80	—	84	—	92	—
May 8	76	95	79.5	89.5	77	96	84	96
June 24	77	84.5	80.5	81.5	82.5	91.5	87	92
July 7	73	84.5	83.5	91	85	91.5	91.5	94.5
Aug. 9	79.5	78	71	87.5	82	90	78.5	91
Sept. 7	84	83.5	84	85	84.5	85.5	87	92
Oct. 7	80	81.5	82.5	80	83.5	85	82	84.5

treated hulless plantings gave 0.33 per cent smutted panicles as compared with 3.53 per cent in the common varieties, while the untreated hulless plantings showed an average of 70.45 per cent smutted panicles as compared with similar controls of hulled varieties showing 23.45 per cent. The average for all treated samples was 2.47 per cent smutted panicles as compared with 39.12 per cent average for untreated samples. The use of 2 oz. copper carbonate per bushel gave practically as good control as larger amounts of the dust. Lambert and Bailey (9) reported tests in Minnesota giving perfect control of oat smut and no seed injury by use of copper carbonate, but our tests have not given such good protection. The protection afforded by the copper carbonate was not equal to that ordinarily obtained with the standard formaldehyde treatments.

DISCUSSION AND SUMMARY

The experiments reported have given very promising results on bunt control from the use of a number of dust treatments, with copper carbonate dust leading in effectiveness. It has been generally conceded that the present methods of seed disinfection for bunt have numerous undesirable features. Some of the features which any other method should have in order to replace the old standard methods are as follows:

1. The elimination of the inconvenient and disagreeable soaking or steeping methods.
2. The elimination of seed injury and retarded growth under conditions of average moisture.

TABLE 8—*Results of control of oat smut using copper carbonate dust treatment*

Treatment	Variety	Average Percentage of smutted panicles for three degrees of smutting	Degree of smutting	Average Percent of smutted panicles for three varieties
Copper carbonate dust 2 oz. per bushel	Swedish Select	2.9	¹ Light	4.13
	Abundance	6.14	Medium	2.45
	¹ Chinese Hulless	0	Heavy	5.5
Copper carbonate dust 3 oz. per bushel	Swedish Select	2.87	² Light	2.8
	Abundance	3.55	Medium	2.79
	¹ Chinese Hulless	0	Heavy	1.73
Copper carbonate dust 4 oz. per bushel	Swedish Select	2.22	² Light	.73
	Abundance	3.55	Medium	2.12
	¹ Chinese Hulless	1.00	Heavy	3.90
Control smutted untreated	Swedish Select	28.12	¹ Light	16.88
	Abundance	18.79	Medium	34.52
	Chinese Hulless	70.45	Heavy	48.16

3. The elimination of danger from seeding in the dry, so as to make possible the seeding of grain in the "dust."

4. The possibility of treating seed days or even months before it is needed for seed, without extra expenditure of labor, thus making possible the utilization of spare time.

5. A better protection from reinfection of treated seed than is afforded by bluestone, especially from wind-borne spores that are generally present in our summer fallow fields.

6. The increase of yields over those obtained with the old methods.

According to the results of our experiments, copper carbonate dust comes nearer to embodying these desired features than any other fungicide. Under no conditions have we found any reduction in germination from the use of this fungicide, either immediately after treatment or after a period of nine months storage. On the contrary there has been an improved germination of treated seed over untreated, resulting under field conditions in better stands, a more rapid germination and a more vigorous growth. Our field records indicate that copper carbonate gives less loss from winter injury and increased yields, although reliable quantitative data on yields are not yet available. As far as

¹Average for Chinese Hulless for medium and heavy degrees of smutting only.

²Average for light degree of smutting for Swedish Select and Abundance only.

seed injury is concerned, seeding in the dry is absolutely safe with copper carbonate, but we do not yet have data on the effectiveness of the fungicide under different conditions of soil moisture. Some of the results point to its greater effectiveness in dry soils.

Copper carbonate has been effective in preventing smut from seed-borne spores as shown by spring seedings, two ounces per bushel of seed giving a smut-free crop in our experiments of 1921 and three ounces per bushel affording perfect protection in 1922. This has been true with light, medium and heavy smutting in which the untreated seed gave the following results:

Degree of smutting	Average percentage of smutted heads from duplicates		
	Marquis	Bluestem	Jenkins Club
Light smutting.....	0.47	6.42	8.35
Medium smutting	0.42	36.33	36.05
Heavy smutting... ..	0.83	58.4	62.48

In the prevention of smut from fall seedings on summer fallow, the results have not been quite so convincing. The field demonstrations in eight counties showed better control with copper carbonate than with either bluestone or formaldehyde, although there were a few specific cases in which bluestone gave less smut. In some local experiments, using three varieties in both smutted and normal summer fallow, the copper carbonate was not quite equal to bluestone but gave better protection than formaldehyde. It is felt that some of the adverse results obtained with copper carbonate were due to inferior brands, and this possibility is being checked by further experiments.

The comparative protection given by the dust fungicides tested when used for heavily smutted spring seedings at the rate of 2 oz. per bushel was as follows:

Fungicides	Percentage of smutted heads		
	Marquis	Bluestem	Jenkins Club
Copper carbonate, pure.....	0	0.89	0.9
Corona compound of copper carbonate.....	0	0.25	0.45
Anhydrous bluestone and calcium carbonate.....	0	0.74	1.15
Anhydrous bluestone and hydrated lime.....	0	3.75	1.79
Seed-O-San.....	0	3.9	1.27

Corona compound of copper carbonate, containing 17 to 18 per cent of metallic copper, gave practically as good protection as the pure copper carbonate, while the much cheaper mixture of anhydrous bluestone and calcium carbonate was not far behind in effectiveness. Our results indicate that less than two ounces of copper carbonate may give protection if in form to insure a perfect distribution over the seed. Our experiments on the comparative effectiveness of these dusts for fall seedings have not yet been completed.

In the treatment of oats for smut (*Ustilago levis*) formaldehyde gave better results than copper carbonate as the average of five farm demonstrations. In our local experiments with three varieties, copper carbonate gave perfect control in Chinese Hulless, but rather poor protection in Swedish Select and Abundance.

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THE GENUS POLYSTICTUS AND DECAY OF LIVING TREES

JAMES R. WEIR

Extensive field observations show that some of the common species of *Polystictus* are not entirely confined to the wood of dead trees. The most common species of the genus found in the United States and the actual cases in which they have been observed to attack the sapwood or heartwood of living trees are as follows:

1. *Polystictus abietinus* (Dicks) Fr.; on the dead wood of blazes, fire scars and frost cracks of *Abies grandis*,¹ *Tsuga heterophylla* and *Thuja plicata*, Idaho, Washington, and Montana. The sapwood is decayed and the adjacent heartwood discolored. The trees were of low vigor. Like conditions have been observed on *Abies balsamea* in northern Minnesota.

2. *Polystictus biformis* Kl., (*P. cervinus* (Schw.) Cke.) on dead wood of living brace roots of *Quercus rubra* and *Fagus atropunicea*, causing a decay of the sapwood and heartwood, extending into the trunk; Indiana and Ohio.

3. *Polystictus cinnabarinus* (Jacq.) Fr.; on the dead wood of a large open wound on living *Betula occidentalis*, the rot extending into the heartwood for several inches beyond the edge of the living sapwood; Idaho.

4. *Polystictus conchifer* (Schw.) Sacc.; common on the dead branches of various species of *Ulmus* killed by shade or other causes. The decay may extend within the living collar of the dead branch from one to three inches. The writer has never observed the fungus to form a heart rot in this position. The fungus is not parasitic and will not directly attack a living branch, although it often appears to do so. Inoculations on healthy branches of *Ulmus* at Great Falls, Va., August, 1921, resulted negatively. Common throughout the Middle West and Eastern United States. Rarely found on the wood of other trees.

5. *Polystictus floridanus* (Berk.) Fr. (resupinate form *Poria lindbladii* Berk.) on the dead wood of wounds in root crotches of living *Liquidambar styraciflua*, Alabama, and on fire scars on living roots of *Chamaecyparis thyoides*, North Carolina, the decay encroaching upon the more vital parts of the roots.

¹ Tree nomenclature according to Sudworth, G. B. Check list of the forest trees of the United States, their names and ranges. United States Dept. Agric., Div. Forest. Bull. 17. 144 p. 1898.

6. *Polystictus hirsutus* (Wulf.) Fr.; causing a sapwood and heartwood rot of living cultivated plum, cherry, prune, peach and apple. Idaho, Washington, Oregon, Indiana. The fungus enters through knots of pruned branches, frost checks and scars made during cultivation. The decay which may be uniform in the heartwood is often serious and results in the trees being broken by the wind. Many small sporophores (4) may appear at the lenticels. The fungus has been observed in the heartwood of living *Alnus tenuifolia*, *Betula occidentalis*, *Acer rubrum*, *Quercus prinus*, *Q. coccinea* and *Castanea dentata*; Idaho, Indiana, Michigan, Tennessee.

7. *Polystictus lacteus* (Fr.) causes a sap and heart rot in living cultivated plum, cherry, peach, and apple. Occasionally the fungus is of considerable economic importance. Zeller (5) states that it causes the most common heart rot of sweet cherry in western Oregon. The fungus enters through pruning scars and other injuries, on street and park trees and occasionally in the forest, it attacks the wood of large wounds and causes a local decay of the heartwood. *Acer*, *Platanus*, *Quercus* and *Ailanthus*; Washington, Oregon, Idaho, Indiana, and Virginia.

8. *Polystictus paryamensis* Fr. This fungus when once established on the dead wood of wounds on living trees may continue to rot the heartwood indefinitely. This condition has been observed on *Quercus alba*, *Q. coccinea*, *Q. marilandica*, *Acer saccharum*, *Betula lutea*, *Fraxinus americana*, *Nyssa sylvatica* and *Populus tremuloides*. Indiana, Tennessee, Michigan, Virginia. Numerous minor cases on various hosts have been observed. Rhoads (1) has called attention to the action of this fungus in several trees.

9. *Polystictus pinsitus* (Fr.) Cke. on dead wood of blazes and open knots of living *Juniperus virginiana* and *Chamaecyparis thyoides* confined chiefly to the surrounding dead sapwood. Virginia and North Carolina.

10. *Polystictus versicolor* (L.) Fr., causes a heart and sapwood rot in living trees of various species. The fungus enters through the dead wood of exposed wounds and may, in some cases, become of economic importance. Von Schrenk (2) and Stevens (3) have fully described the damage caused by this fungus in Catalpa. Ordinarily the fungus is of little importance on living trees.

11. *Polysticus zonatus* Fr., a well defined species and especially abundant in the western United States, occasionally causes a heart rot in living trees of *Populus* and *Salix*. The fungus enters through the exposed wood or wounds. Notable cases are recorded in Idaho, Washington and in the Black Hills of South Dakota.

With the exception of the purple pored species, numbers 1, 5, and 8, all of which cause a characteristic rot with small, elongated pockets, the rots of the majority of the species mentioned are not especially differentiated. The character of the decay, however, may be influenced slightly by the species of tree structure and the physical nature of the wood. This may be observed when any of the above named species which normally grow on the wood of frondose trees attack the wood of conifers, an occasional occurrence, or in a change from porous to non-porous wood. The general color of the decays of all the species mentioned is usually white or yellowish or of a slight reddish cast in the case of *Polystictus cinnabarinus*, due to the massing of hyphae near the sporophore.

In but few cases have the *Polystictus* rots been found to be of much importance on forest trees and may be disregarded in the management of original forest lands. Usually dependent upon the presence of a large amount of weathered dead wood to effect encroachment on the heartwood or the adjacent sapwood, the decay of most species makes slow progress in otherwise healthy trees. Trees whose vigor is reduced by extensive injuries involving a large part of the side of the trunk or the brace roots may suffer greater damage. As a rule, these fungi are not to be considered in the same class with the true destructive cull fungi of living timber trees. They are primarily the scavengers of the forest, decaying the slash of timber sale areas, and other debris, but may become important in the decay of useful material too long left in the woods or in hastening the decline of street and park trees.

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PHYTOPATHOLOGICAL NOTES

Poria papyracea (Schw.) Cke.—This rare and interesting species has been recently collected by the writer as an epiphyte on the thin, smooth chlorophyllaceous bark of *Chamaecyparis thyoides* in the Dismal Swamp of Virginia. The area of bark over which the fungus extended was browned and somewhat sunken below the general level, but the vital parts were not affected. The fungus has also been found on dead fallen twigs and branches of *Thuja occidentalis* by J. J. Neuman at Crandon, Wisconsin, and by Dr. A. H. W. Povah at Vermilion, Michigan. Good collections in the N. Y. Botanical Garden were made by Underwood on *Vitis* at Auburn, Alabama. The original specimen was collected by Schweinitz on dead bark of *Vitis* in Carolina. It was later collected by Peters (464) on *Vitis* in Alabama, and described by Berkeley and Curtis under the name of *Polyporus barbaeformis* (Poria, Sacc.). The habit of growing on smooth green bark is similar to that of *Septobasidium retiforme* (Berk. & Curt.) Pat. and *S. frustulosum* (Berk. & Curt.) Pat., the latter being very common on the bark of young ash in Mexico and is considered injurious.

Poria papyracea is easily distinguished from all other members of the genus by its broad elongate elliptic or cylindric guttulate spores with the appearance of peculiar longitudinal striations or reticulate echinulations; the spores average $15-16 \times 5.9-8.5 \mu$.—JAMES R. WEIR.

REPORT OF THE FOURTEENTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The fourteenth annual meeting of the Society was held in the Main Building of the Massachusetts Institute of Technology, Cambridge, Massachusetts, December 27-30, 1922, in conjunction with the American Association for the Advancement of Science and affiliated scientific societies. President E. C. Stakman of Minnesota presided and about ninety-five members were present. The headquarters of the Society were at the Parker House, Boston, Massachusetts.

There were seventy-nine papers on the regular program, abstracts of which were printed in the January number of *Phytopathology*. Advance separates of the abstracts were distributed for use at the meetings.

There were three joint sessions with other societies. On Wednesday afternoon, December 27, occurred the annual meeting of Section G of the American Association for the Advancement of Science. At that meeting Dr. Mel. T. Cook, retiring Vice President of Section G, gave an address entitled "The Origin and Structure of Plant Galls." This address was followed by a symposium on Sterility of Plants, papers being given by A. B. Stout on Physiology of Incompatibility, B. M. Davis on Pollen and Seed Sterility in Hybrids, E. M. East on Genetical Aspects of Self and Cross Sterility, and M. J. Dorsey on Sterility in Relation to Horticulture. On Saturday morning, December 30, a joint session was held with the American Association of Economic Entomologists, the subject being a symposium on Plant Quarantines. Papers were presented by C. L. Marlatt on The When and Why of Plant Quarantines, W. A. Orton and R. K. Beattie on Scientific Basis of Foreign Plant Quarantines, W. A. McCubbin on Factors Contributing to Success in Domestic Quarantines, Wilmon Newell on Tropical and Sub-tropical Quarantines, and Lee A. Strong on Western Views on Plant Quarantines.

On Thursday morning the American Phytopathological Society joined the Physiological Section of the Botanical Society of America in a symposium on Mosaic Diseases of Plants, at which fourteen papers were presented. The meeting was noteworthy in that four papers reported the finding of definite bodies or organisms in the tissue of plants affected with mosaic. L. O. Kunkel reported his further studies on the intracellular bodies associated with mosaic of *Hippeastrum equestre* and corn in Hawaii. H. H. McKinney, Sophia H. Eckerson and R. W. Webb gave two papers dealing with the intracellular bodies associated with mosaic of *Hippeastrum johnsonii* and also with the rosette disease of wheat, while Ray Nelson, in a paper illustrated by beautiful photomicrographs, reported the finding of definite flagellate protozoans constantly associated with leaf roll of potato and with mosaic of bean, clover and tomato. It seems probable that the intracellular bodies reported by Kunkel and by McKinney, Eckerson and Webb are also protozoans. Further studies in which the best modern protozoan technique is used should yield results of the greatest importance in adding to our knowledge of the cause of this type of disease. Without doubt this symposium marks an important milestone in the progress of plant pathology.

The annual dinner of the Society was held on Friday evening, December 29, in the Walker Memorial Building of the Massachusetts Institute of Technology and was attended by one hundred and sixteen pathologists and botanists.

The following officers were elected:

President, G. R. Lyman, West Virginia College of Agriculture, Morgantown, W. Va.

Vice President, F. D. Fromme, Virginia Polytechnic Institute, Blacksburg, Va.

Secretary-Treasurer, R. J. Haskell, U. S. Department of Agriculture, Washington, D. C.

Councilor for two years, M. F. Barrus, New York State College of Agriculture, Ithaca, N. Y.

Councilors for one year (chosen by the Divisions of the Society), W. P. Fraser, University of Saskatchewan, Saskatoon, Saskatchewan, representing the Canadian Division; F. D. Heald, State College of Washington, Pullman, Wash., representing the Pacific Coast Division; and L. R. Hesler, University of Tennessee, Knoxville, Tenn., representing the Southern Division.

Members of the Editorial Board of Phytopathology (chosen by the Council of the Society): Editor for one year, N. J. Giddings, West Virginia University, Morgantown, West Virginia; Associate Editors for three years, J. G. Leach, College of Agriculture, University Farm, St. Paul, Minnesota; W. D. Valleau, University of Kentucky, Lexington, Kentucky; R. D. Rands, U. S. Department of Agriculture, Washington, D. C.; W. A. McCubbin, Bureau of Plant Industry, Harrisburg, Pennsylvania; Business Manager for one year, R. J. Haskell, U. S. Department of Agriculture, Washington, D. C.; Advertising Manager for one year, R. G. Pierce, U. S. Department of Agriculture, Washington, D. C.

Member of the Editorial Board of American Journal of Botany for three years (chosen by the Council): Donald Reddick, Cornell University, Ithaca, N. Y.

Representative on Board of Control of Botanical Abstracts for four years (elected by the Society): E. C. Stakman, University of Minnesota, University Farm, St. Paul, Minnesota.

Representative on the Council of the American Association for the Advancement of Science for one year (chosen by the Council): G. P. Clinton, Agricultural Experiment Station, New Haven, Connecticut, and W. A. Orton, U. S. Department of Agriculture, Washington, D. C.

Members of the Advisory Board (chosen by the Council): C. W. Hungerford, University of Idaho, Moscow, Idaho, for three years to succeed H. P. Barss as Commissioner for the West; L. R. Hesler, University of Tennessee, Knoxville, Tennessee, for three years to succeed J. A. Elliott as Commissioner for the South; L. R. Jones, University of Wisconsin, Madison, Wisconsin, for three years, and M. F. Barrus, Cornell University, Ithaca, N. Y., for two years, to succeed L. R. Jones and E. M. Freeman, respectively, as Commissioners at Large.

REPORT OF THE TREASURER FOR 1922

Receipts:

Balance from 1921	\$1,009.90
Annual dues	3,757.86
Subscriptions received with annual dues	5.00
Contribution to Phytopathology deficit received with annual dues	3.00
Excess dues	5.60
Interest on checking account	36.95
Exchange on foreign membership checks	.94

Sales received in checks with annual dues	14.50	
Subscriptions to the Oberly Memorial Fund received with annual dues	47.48	
		\$4,881.23

Expenditures:

Member subscriptions transferred to Phytopathology	\$1,792.00	
Secretarial work	250.43	
Stationery, stamped envelopes, printing	215.78	
Subscriptions received with dues transferred	5.00	
Contribution to deficit received with dues	3.00	
Sales received with dues	15.00	
Postage stamps	7.50	
Excess dues returned	5.75	
Duplicate dues returned	4.00	
Secretary-Treasurer's travel and miscellaneous expenses	126.77	
Supplies, rubber stamps, etc.	7.22	
Checks returned by bank	16.25	
Cost of Russian cable	4.80	
Error in discount	.95	
Amount transferred to sinking fund for investment	1,100.00	
		\$3,554.45

Balance	\$1,326.78
Amount of receipts for 1923 and 1924 included above	\$1,027.75

Sinking fund:

Balance due for 1921	\$20.58	
Balance due for 1922	5.00	25.58
		\$1,053.33
Actual balance for the year 1922		\$273.45

In accordance with directions given at the Toronto meeting, the Secretary, in sending out the notices of annual dues for 1923, requested each member to add twenty-five cents to his check as a personal contribution to the fund for the Eunice R. Oberly Memorial Prize. Many members are responding to this request, and \$47.48 have already been received. Full report must be deferred until next year.

On December 20, 1922, the Society had 553 members in good standing:—89 life sustaining and 464 regular, as opposed to 539 in 1921, 495 in 1920, and 452 in 1919. Therefore it is seen that the Society is maintaining a steady growth. Thirty-three candidates were elected to membership.

REPORT OF THE BUSINESS MANAGER OF PHYTOPATHOLOGY FOR 1922

Receipts:

Balance from 1921	\$1,728.14
Member subscriptions transferred from Society	1,792.00

Subscriptions.....	2,013.00
Sales.....	330.15
Advertising, 1921.....	322.54
Advertising, 1922.....	336.53
Contributions to Phytopathology deficit.....	11.24
Interest on invested sinking fund.....	120.00
Balance due sinking fund to June 1.....	1,000.00

\$7,662.60

Expenditures:

Manufacturing Phytopathology:

Vol. XI, No. 8.....	\$343.43
Vol. XI, No. 9.....	234.92
Vol. XI, No. 10.....	307.09
Vol. XI, No. 11.....	268.00
Vol. XI, No. 12.....	308.84
Vol. XI, Index.....	148.72
	\$1,611.00
Vol. XII, No. 1.....	385.41
Vol. XII, No. 2.....	376.13
Vol. XII, No. 3.....	394.58
Vol. XII, No. 4.....	378.61
Vol. XII, No. 5.....	295.12
Vol. XII, No. 6.....	385.29
Vol. XII, No. 7.....	185.05
Vol. XII, No. 8.....	367.35
Vol. XII, No. 9.....	364.56
	\$3,132.10

\$4,743.10 \$4,743.10

Miscellaneous Journal expenses (Dix lists, etc.).....	\$86.85
Secretarial work.....	74.28
Supplies, miscellaneous.....	7.10
Rebate for parts of journal not supplied.....	4.00
Printing bill heads.....	10.00
Postage.....	33.18
Duplicate subscription returned.....	5.00
Miscellaneous expenses of Business Manager.....	39.45
Miscellaneous expenses of Editor-in-Chief.....	18.24
Checks returned by bank.....	10.75
Check to reimburse G. R. Lyman for amount paid from his personal account in error.....	5.00
Sinking fund invested with accrued interest.....	1,002.72
Refund on cancelled order.....	1.65
	\$6,041.32

Balance.....	\$1,621.28
Amount of 1923 subscriptions included in the above receipts.....	303.15

Actual balance for 1922..... \$1,307.63

Sinking Fund:

Amount invested in first mortgage notes	\$3,000.00
Amount invested at 4% time deposit for 6 months	100.00
Balance in Society treasury due sinking fund 1921-22	25.58
	\$3,125.58

At the Chicago meeting two years ago the business manager reported an indebtedness of Phytopathology amounting to \$2,179.72. At that time the Society appropriated \$575.00 from its treasury and the members voluntarily contributed \$1,059.72 toward paying this deficit, making a total of \$1,634.72. There still remained an indebtedness of \$545.00, which Phytopathology had to carry as it entered on the new plan of operation in January, 1921.

Two years have now elapsed under the new plan of operation, which included a change of printers, the collection of subscriptions by the business manager, and the appointment of an advertising manager to handle advertisements,—both subscriptions and advertising having been handled previously on a percentage basis by the Williams & Wilkins Company, the former publishers. During the past two years the receipts for both subscriptions and advertisements have increased materially and this increased income, together with reduced expenses, has enabled Phytopathology to overcome the \$545.00 deficit, to increase the number of printed pages per year, and to accumulate a balance, so that if Phytopathology were to suspend publication at the close of 1922 and all bills payable and receivable were liquidated there would be a clear balance of \$892.81. The official journal of the Society has passed its financial crisis safely and is now on firm ground.

An auditing committee consisting of F. D. Fromme and A. T. Evans was appointed to examine the accounts of the treasurer and business manager. This committee found the accounts correct and the reports were adopted.

REPORT OF EDITOR-IN-CHIEF OF PHYTOPATHOLOGY

The printers' strike caused considerable delay in publishing the earlier numbers of Phytopathology during 1922. In spite of this fact, the December number, including the index of the twelve numbers, appeared in January. During 1923 the numbers should come out about on time. It will be noted that several papers somewhat longer than usual have been published during 1922. The editor adopted the policy of accepting or rejecting papers wholly on their merit, regardless of length. There have been published in all during 1922, 597 pages of text, 113 text figures, and 30 full page plates. The abstracts of papers presented at the meetings of the three Divisions, as well as at the Toronto meeting of the Society, have been published during the year. These abstracts, of which there were 145, required 51 pages of text.

REPORT OF THE RETIRING REPRESENTATIVE ON THE BOARD OF CONTROL OF BOTANICAL ABSTRACTS

Dr. Donald Reddick, the retiring representative of the Society, served for two years as Chairman of the Board of Control, has been a member of the Executive Committee for four years and recently has assumed business management of the journal. He submitted the following report:

The problems of the Board have been numerous and varied. The financial problem, however, has and still does overshadow all others. Much time has been given to this all-important phase of the work.

Aside from the more general duties on such a Board your representative has attempted to further the interests of the Society in every legitimate way. He, himself, acted as editor for the section *Pathology* for a short time. An effort was made to liberalize the field of this section by including essential work in general pathology. When the collecting of abstracts was placed in the hands of a bibliographer and the "journal basis" adopted it proved rather difficult to find a suitable plan for accomplishing this. The problem was passed on to Dr. G. H. Coons who made some progress with it, but Doctor Coons was forced to resign and Mr. F. V. Rand now labors with it. The Board has given very great latitude to the editors in the development of their respective sections. It is to be hoped that Mr. Rand will find a way of developing the section on Pathology to its greatest usefulness.

The most regrettable feature of the work has been the general lack of constructive criticism, or, indeed, of criticism of any sort, from members of the Society. In anticipation of just this condition, among others, the Board adopted the working rule that members of the Board shall not be eligible for immediate reelection. The Board, *per se*, cannot enforce such a rule, but if the constituent societies can be induced to observe it, the Board can be maintained as a democratically constituted body and by proper elections our Society can be assured that our best talent eventually will be directed to making *Botanical Abstracts* the best and most complete publication in existence for abstracting pathological articles.

The willingness of members of the Society to support *Botanical Abstracts* has been most gratifying. The response to every appeal for help has been most satisfactory. At least 80 of our members serve as collaborators, many of our members own bonds of the corporation and an unusually large proportion of our members subscribe for the journal.

UNION OF AMERICAN BIOLOGICAL SOCIETIES

At the Toronto meeting this Society gave preliminary consideration to this subject and designated Donald Reddick and G. R. Lyman as its representatives at a proposed inter-society conference to consider a plan of federation. This conference was held in Washington last spring and all the representatives of the biological societies present were unanimously in favor of forming a federation. Therefore, an executive committee was appointed to draft a constitution and to work out the details of organization. The report of this executive committee, giving the wording of the proposed constitution, was printed in *Science* (*Science* LVI: 359-361, Sept. 29, 1922) and reprints of this article were distributed to all members of the American Phytopathological Society in advance of the Boston meeting. Membership in the proposed union is by societies, not by individuals. Each member society retains complete autonomy and is entitled to two representatives on the Council of the Union. The principal primary purpose of the Union is the promotion of biological publications.

After consideration, the Society voted to accept membership in the proposed Union of American Biological Societies and requested the Council of the Society to select the two representatives on the Council of the Union. Donald Reddick and Perley Spaulding were named by the Council as the representatives of the American Phytopathological Society.

REPORT OF THE ADVISORY BOARD

The Advisory Board reported to the Council through its Chairman, C. R. Orton. The report was referred to the Society by the Council and was adopted as follows:

Summer conference:—The fourth annual summer meeting of the Society was held in Delaware, New Jersey, and Philadelphia and vicinity, being devoted to the diseases of vegetables and the problems of market pathology. The meetings were attended by sixty-five scientists besides a considerable number of growers and others interested in marketing problems. Members were present from the Philippine Islands, Ontario, Quebec, Nova Scotia and twelve states. There were present as guests of the Society, Dr. I. B. Pole-Evans, Pretoria, South Africa; Senor Alfonso Madariaga, Director of Agriculture, San Jacinto, Mexico; Dr. C. Boyle, Dept. of Agriculture, Dublin, Ireland; Dr. Seiya Ito, Hokkaido Imperial University, Sapporo, Japan; Dr. Takewo Hemmi, Kyoto Imperial University, Kyoto, Japan; Dr. D. N. Borodin, Russian Agricultural Bureau, 136 Liberty Street, New York City; Dr. Rudolf Kuraz, Agricultural Attache, Czechoslovak Legation, Washington, D. C.; Mr. Carlos Vallejo, Agricultural Attache, Argentine Legation, Washington, D. C.; and Mr. R. J. Noble, Dept. of Agriculture, New South Wales, Australia. A more extended report of the conference, prepared by the Secretary, Dr. R. J. Haskell, has already been published in *Phytopathology*.

Phytopathological project list:—At the Toronto meeting it was decided to discontinue this list as an annual project. There is some doubt whether such a list is worth the time and effort necessary to prepare it. By request of the Advisory Board, a vote of the Society was taken at Boston to ascertain whether this list should be continued, and a large majority of those present voted that it is inadvisable at present to continue the preparation of such lists.

Phytopathological Institute:—Dr. E. C. Stakman was chosen leader of this project at the Toronto meeting. Certain phases of co-operative researches on mosaic have been undertaken at the Thompson Institute, and at the summer conference in Philadelphia Dr. William Crocker presented to the Advisory Board the plans for that Institute and requested the counsel of the Advisory Board regarding the development of work at the Institute along phytopathological lines. Everything possible will be done to facilitate the development of such work at the Thompson Institute.

Some progress has been made in eliciting the interest of officials in the U. S. Department of Agriculture toward offering their facilities for co-ordinating certain types of research work, and providing space and other facilities for visiting scientists.

Cooperation with the National Research Council:—The Advisory Board functions as the Committee on Co-operation in Phytopathology of the National Research Council. The Chairman is also a member of the Division of Biology and Agriculture, succeeding Dr. G. R. Lyman whose term of office expired June 30, 1922. In this capacity the Board has fostered a number of co-operative projects. During the past year special attention has been given the Arthur rust project, dusting of fruits and vegetables, seed borne diseases, use of sulphur for control of potato scab, sulphur investigations, nickel investigations, Scalecide investigations, and cereal seed disinfections. All but the first mentioned are co-operative with the Crop Protection Institute.

The Rust project was initiated September 1 through the joint efforts of the Division of Biology and Agriculture of the National Research Council; the Office of Cereal Investigations, U. S. Department of Agriculture; the officers of the Indiana Agricultural Experiment Station, the Pennsylvania State College, and the Virginia Polytechnic Institute; and the Arthur Committee. Laboratory space and facilities, a clerical graduate assistant, library space, and the time of certain investigators has been provided by the Indiana Agricultural Experiment Station; travelling expenses of co-operators are being provided by the National Research Council; an honorarium for Dr. Arthur was provided by the Office of Cereal Investigations, U. S. Department of

Agriculture; and the Pennsylvania State College and Virginia Polytechnic Institute have agreed to grant leave of absence with pay to F. D. Kern and C. R. Orton of the former and F. D. Fromme of the latter to aid Dr. Arthur in the preparation of the manuscript of a volume on Plant Rusts. The Indiana Experiment Station has also agreed to grant time to H. S. Jackson and E. B. Mains for a similar purpose. The co-operators on this project met at Lafayette in June and drew up a tentative outline on Plant Rusts and steady progress has since been made by Dr. Arthur with the assistance of the graduate student provided.

The dusting project has been under the leadership of F. D. Fromme, N. J. Giddings and P. J. Parrott during the past year. Co-operative tests have been conducted in several states and some Canadian provinces, and some noteworthy results obtained.

The project on seed borne diseases has been handled in co-operation with the Crop Protection Institute under the leadership of a special committee composed of M. F. Barrus, M. T. Munn and the Chairman of the Advisory Board. A preliminary list of seed borne diseases has been prepared. The subject was presented by C. R. Orton before the American Seed Trade Association at their annual meeting in Chicago, June 22. Action has been taken by the Association of Official Seed Analysts leading to the practice of reporting disease indications upon the certificate as a part of the service rendered. Good progress is being made on this project and assurance has been given that the matter will receive special attention by the Executive Committee of the American Seed Trade Association. The committee is to be continued.

The project on use of sulphur for the control of potato scab has been under the leadership of C. R. Orton and G. H. Coons.

The sulphur investigations have been under the leadership of a committee composed of G. H. Coons, P. J. Parrott and C. R. Orton. Fifteen thousand dollars have been subscribed during the past year by three sulphur producing companies for fundamental researches upon the effect of elemental sulphur upon fungi and insects. Two investigators have been secured, one of whom is working under the direction of Dr. B. M. Duggar at the Missouri Botanical Garden and the other is at work under the joint leadership of Professor Herbert Osborne at Ohio State University and Dr. P. J. Parrott at the New York Agricultural Experiment Station. Important results have already been secured and this project is well established.

The nickel investigations project is of a preliminary nature and no attempt has been made to expand it beyond these preliminary tests up to the present time. Co-operative tests have been made in Delaware, Pennsylvania, Minnesota, Quebec, and Ontario during the past year.

The B. G. Pratt Company has recently signed a contract pledging forty-five hundred dollars annually for a period of not less than three years for the purpose of investigating the effects of Scalecide upon fire blight and upon the rate of growth of apple and pear trees. This project is under a committee composed of C. R. Orton, H. W. Anderson and P. J. Parrott. The funds are to be made available January 1, 1923, and investigations will be started this spring.

The cereal seed treatment project under the leadership of C. R. Orton, E. C. Stakman and E. B. Lambert, was initiated in the spring of 1922 in several states and Canadian provinces. The copper carbonate has been furnished through the Crop Protection Institute and tested out on wheat and oats along with copper sulphate and formaldehyde. The results to date have been collected in mimeographed form.

"Science Service" is in need of promotion by the Society and to this end the Secretary, Dr. Haskell, has been giving it personal attention. The Advisory Board will endeavor to secure the interest of other workers.

Crop Protection Institute:—Two members of the Advisory Board, G. H. Coons and C. R. Orton, have served during the past year upon the Board of Governors of the Crop Protection Institute. It seems important that these two agencies should be co-ordinated by the continuance of this policy. A considerable amount of time has been devoted by the various members of the Board of Governors to the Crop Protection Institute and this must continue throughout the early years of its development. C. R. Orton, whose term expired at the Boston meeting, was elected to succeed himself for a three year's term. F. D. Fromme is the third representative of the Society upon the Board of Governors.

REPORTS OF COMMITTEES

The Committee on Standardization of Biological Stains:—Dr. R. H. Colley made a brief report on data obtained from a test questionnaire sent to members of the Society. The conclusions reached were that most of the laboratories of all plant pathology are still supplied with European stains and that success with American stains depends largely upon the skill of the operator in handling new products. Members of the Society interested in the work of standardization of American biological stains were urged to communicate personally with Dr. H. J. Conn, Geneva, N. Y. The report was accepted and the committee discharged.

The Committee on the F. Kolpin Rawn Fellowship reported through its chairman, Dr. L. R. Jones, that further inquiries have been made looking toward the endowment of a Fellowship, but that no definite progress could be reported as yet. On recommendation of the committee the report was accepted and the committee discharged. On motion of Dr. Jones it was voted that the Council appoint a new committee to carry on this work, and such a committee was appointed, consisting of R. Kent Beattie, A. G. Johnson and C. L. Shear.

The Committee on Bacterial Nomenclature:—Dr. Erwin F. Smith, chairman of this committee, stated that no formal report could be made at the Boston meeting. The committee was continued.

PHYTOPATHOLOGICAL RESEARCH IN THE TROPICS

W. A. Orton reported on this subject as chairman of a special committee of the National Research Council. This committee has been working in co-operation with the Advisory Board and has developed plans looking toward the incorporation of an organization to be called "The Tropical Phytopathological Institute." This institute is designed to provide an efficient and reliable organization for the advancement of knowledge of the native plants and cultivated crops of the tropics, including their diseases and other pests. The plans of the committee were approved in principle by the Society and the committee was instructed to proceed with the development of the plans and to secure the endorsement of the National Research Council.

RESOLUTIONS

A committee on resolutions, consisting of L. M. Massey, F. L. Drayton and H. W. Anderson, was appointed by the president. This committee reported the following resolutions, which were unanimously adopted.

1. The American Phytopathological Society wishes to express to Dr. W. H. Weston and the other members of the local committee on arrangements, to the authorities of the Massachusetts Institute of Technology, and to the municipal authorities of Boston and Cambridge its sincere appreciation of the courtesies and hospitality extended to its members throughout the meetings of the Society.

2. The American Phytopathological Society wishes to place on record its appreciation of the liberal assistance given by the National Research Council to the Board of Control of Botanical Abstracts which has made possible the continued publication of this journal.

3. In view of the efficient and untiring services rendered by our retiring secretary and treasurer, Dr. G. R. Lyman, at great personal sacrifice, the members of the American Phytopathological Society assembled at their annual meeting in Boston wish to express their sincere gratitude. As business manager of Phytopathology, Doctor Lyman, by his unceasing efforts, has rendered plant pathologists a lasting service by maintaining the journal at a high standard of efficiency.

4. The American Phytopathological Society in annual convention assembled expresses its deep regret at the passing on October 29, 1922, of Dr. Elias J. Durand, professor of botany at the University of Minnesota. Dr. Durand has been active for many years as a teacher of botany and as an investigator, especially in the field of mycology. The Society extends sympathy to his many friends, especially to his family and to his co-workers in the University of Minnesota.

5. It is with profound sorrow that we, the members of the American Phytopathological Society in annual conference assembled have to record the death, on June 13, 1922, of Frederick C. Werkenthin, assistant professor of botany at the Iowa State College of Agriculture, Ames, Iowa. Professor Werkenthin was most successful as a teacher, commanding the respect and confidence of students and faculty at all of the institutions where he served. He showed unusual promise as an investigator and during his brief scientific career he made a number of noteworthy contributions to botanical science. On account of this, our mutual loss, the American Phytopathological Society wishes to express the deepest and most sincere sympathy of its members to the family, colleagues and friends of our departed brother.

FOREIGN MEETINGS

At the annual dinner, Dr. W. A. Orton presented an invitation received from Dr. H. M. Quanjor for the American Phytopathological Society to be represented at the International Conference of Phytopathology and Economic Entomology in Holland, June, 1923. The conference will convene at Wageningen on Sunday, June 24, and will adjourn June 30 at The Hague. Various important agricultural districts of Holland will be visited and attention will be given to diseases of potatoes, fruit and truck crops, flower bulbs and ornamentals. President Stakman also spoke briefly of the Pasteur Centennial celebration in the form of a Scientific and Industrial Exposition of Hygiene at Strasbourg, June to October, 1923, which Americans are urged to attend and to which they are invited to send exhibits. The Society expressed its appreciation of these invitations and instructed the Council to do all in its power to promote the attendance of several of our members as accredited representatives of the Society.

MISCELLANEOUS BUSINESS

The secretary's report of the thirteenth annual meeting held at Toronto, Canada, December 27-31, 1921, was adopted without correction as published in No. 4, volume 12 of *Phytopathology*.

The Society voted to hold its fifteenth annual meeting at Cincinnati, Ohio, December 27, 1923, to January 2, 1924, in conjunction with the American Association for the Advancement of Science and other biological societies.

At the Toronto meeting President Reddick was authorized to send a contribution of \$25.00 in the name of the Society to a memorial in Rome, to Dr. Giuseppe Cuboni, late

Director of the Racle Stazione di Patologia Vegetale. Dr. Reddick reported that the principal phytopathological centers in America had been asked for contributions to this fund and the response had been so generous that a total of \$36.50 was received. Of this amount \$25.00 was sent to Rome and the balance, \$11.50, was added to the Oberly Memorial fund. Dr. Reddick read to the Society the letter of appreciation of this gift received from Professor Traverso.

At the annual meeting of Plant Disease Survey collaborators it was voted to recommend to the American Phytopathological Society that a committee of the Society be appointed to carry on a campaign of education looking to more adequate financial support of the Plant Disease Survey. The Society approved this recommendation and directed the president to appoint the chairman of such a committee who should be authorized to select the other members of the committee. President Stakman appointed Dr. N. J. Giddings as chairman of this committee.

At the annual dinner the matter was discussed of sending *Phytopathology* to pathological workers in certain European countries where the exceedingly unfavorable rates of exchange make subscription to the journal impossible. Dr. Stakman and Dr. Spaulding, who have recently returned from European trips, spoke of the deplorable lack of current pathological literature in the libraries of our colleagues in these countries and urged members of the Society to adopt a more liberal policy in sending their separates abroad. After discussion, the members present voted unanimously to raise money to defray the expense of sending *Phytopathology* to foreign pathologists. The hat was passed and \$105.00 in cash was collected. It was voted that this money be used to send *Phytopathology* to institutions and to certain individuals selected by the Council. It was further voted that the Council be authorized, after investigation, to complete gratis the files of *Phytopathology* in certain institutions, especially in Austria, Hungary, Germany and Russia, where in the judgment of the Council complete sets will be of greatest value.

G. R. LYMAN, *Secretary.*

The March number of Phytopathology was issued March 18, 1933.

PHYTOPATHOLOGY

VOLUME XIII

NUMBER 5

MAY, 1923

STUDIES ON THE BLIGHT OF CUCURBITS CAUSED BY MACROSPORIUM CUCUMERINUM E. & E.

HAROLD ROY BRISLEY¹

WITH THREE FIGURES IN THE TEXT

A leaf blight of cucurbits, caused by *Macrosporium cucumerinum* E. & E. is of great economic importance in the sections of Arizona where cucurbits are grown for commercial purposes. The disease has progressed so rapidly from year to year that during 1921 the crops of cantaloupes and watermelons were almost a complete failure in some sections. The failure was largely due to the leaf blight, although such other diseases as anthracnose (*Colletotrichum lagenarium* (Pers.) E. & H.) and a bacterial wilt were present.

SYMPTOMS

The disease usually starts about the middle of the growing season. The first noticeable evidence of it is a tan colored spot the size of a pin head formed when the organism has invaded the leaf tissue of the host. These minute spots enlarge and merge together, ultimately killing the leaf. With the leaves thus affected, a field of melons appears as shown in figure 1. The enlarged spots are made up of concentric, darker rings, always with a small, tan spot at the center. The central spot marks the point of infection, and is characteristic of the blight. The color of the spots varies according to the host, being light brown on cantaloupe and dark brown or almost black on watermelon, but the infection point at the center remains quite constant in color. When the over-ripe fruit is allowed to remain in the field, sunken spots from one-fourth to one inch in diameter appear which later become covered with a dark olive green growth of spores. During several seasons of observations over an extensive area the writer has never seen infections on the stems

¹ Studies here reported are an abridgment of work submitted at the University of California as a thesis for the Master's degree in Plant Pathology in 1922. Acknowledgment is due to Professor W. T. Horne, under whose direction the work was done, for helpful suggestions and criticisms.

or roots, nor on melons growing on diseased vines until the fruit has become over-ripe.

PATHOGENICITY

The organism was readily isolated on prune agar poured plates. The disease was reproduced on squash and cucumber leaves by a series of sixteen inoculations. These were made both by needle pricks and by placing spore-contaminated water on the unbroken leaf surfaces. With a single exception all of the inoculations on the leaves were successful while not a single infection was produced on the stems. The spores produced on one of the diseased leaves were used for making pure

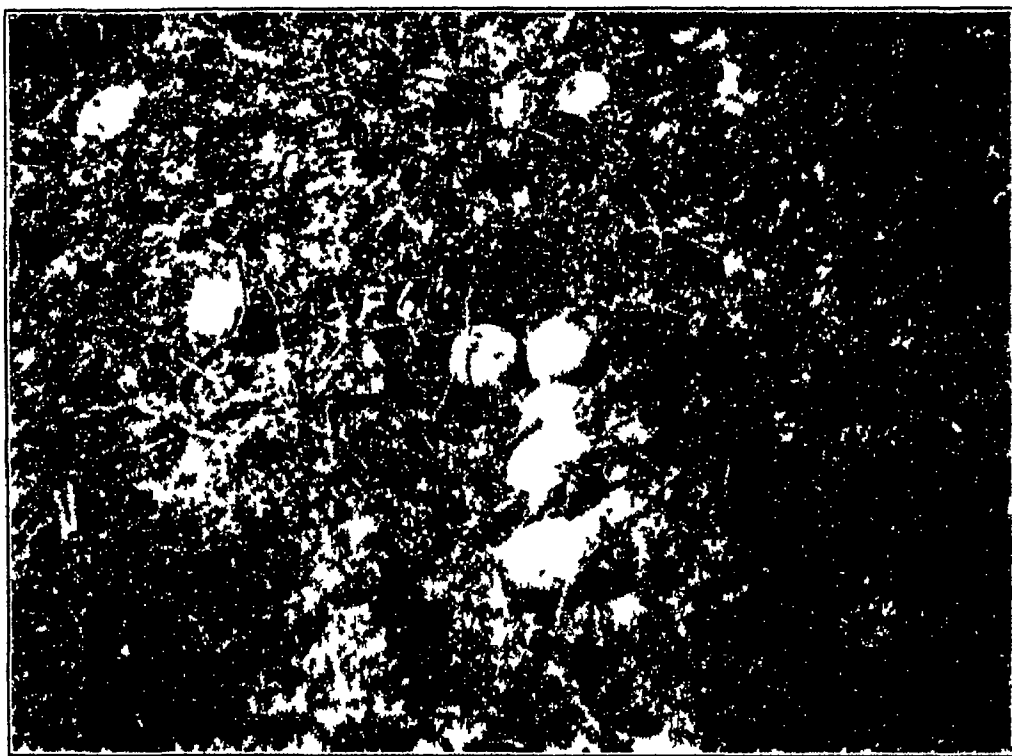


FIG. 1. Leaf blight on cantaloupes.

culture reisolations. One strain was the material employed in making 106 inoculations on cucurbits and 52 on other plants. This large number was made in order to demonstrate the pathogenicity of the organism. Ninety-six per cent of the cantaloupe inoculations, ninety-five of the squash, ninety-two of the watermelon, seventy of the cucumber, fifty of the potato and forty of the tomato were successful. Figure 2 shows infections produced by inoculations on the unbroken surface of a squash and a watermelon leaf. All of the inoculations made on the stems, petioles and roots were unsuccessful.

HOST RANGE AND RELATIVE SUSCEPTIBILITY

Experiments were made with ten different host plants (representing five different families), and it was found that they could be arranged in the following order in respect to susceptibility: cantaloupe, squash, watermelon, cucumber, potato and tomato. Those upon which the organism did not produce blight spots were turnip, cabbage, bean and orange. The growth of blight spots on all of the cucurbits was good, the inoculation period ranging from three to twelve days. It was very slow and uncertain in the cases of the potato and tomato, the period ranging from twelve to fifteen days.

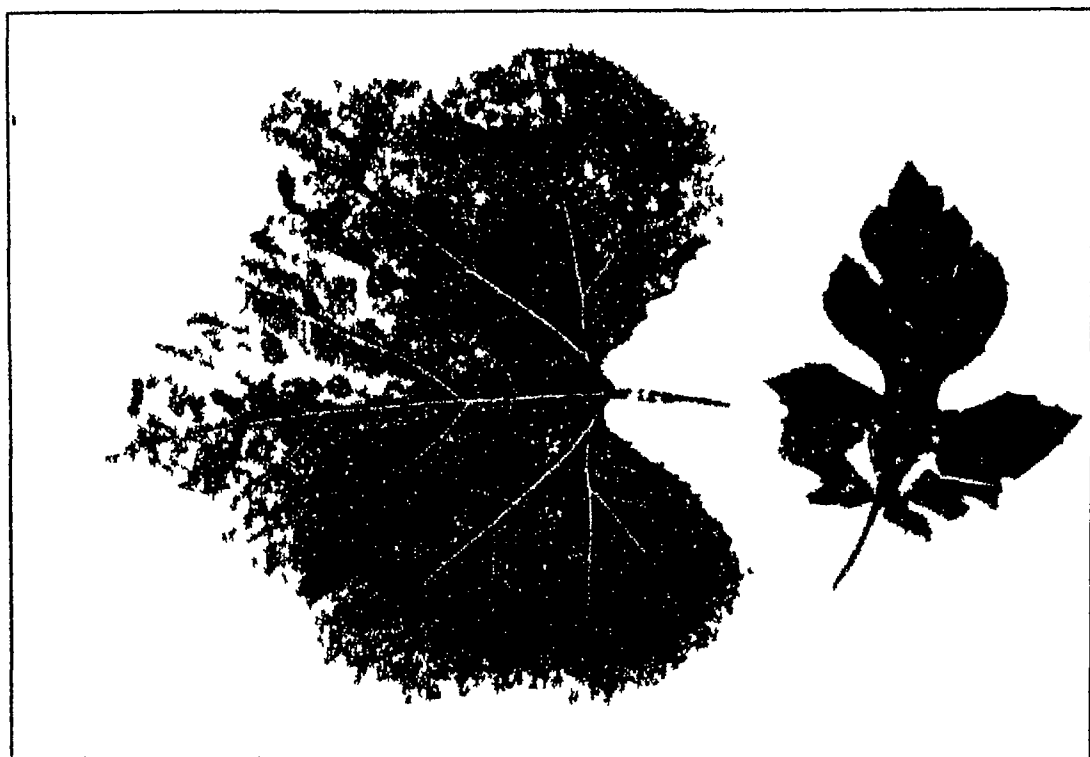


FIG. 2. Cantaloupe leaf-blight from inoculation

CHARACTERISTICS OF THE FUNGUS

Some cultural characters. The organism will grow on any ordinary culture medium, though it grows best and most rapidly on prune agar. In an alkaline or a neutral medium the growth is slow, but when the medium is slightly acid it makes fast progress. The colony is most easily recognizable when grown on gelatin which is liquified at the center of the colony in twelve days. The growth on nutrient agar is also striking as the strands of mycelium cling together to form radiating cords which branch at relatively long intervals. On culture media the colony

is most often of an olivaceous color, which is produced by the mycelial threads and spores. The former grow through the media while the latter are produced on the surface. Many colonies have a large amount of aerial mycelium which appears in cottony masses above the media.

Overwintering of the fungus. Studies described below indicate that the spores of *Macrosporium cucumerinum* do not carry the disease over the winter. This function is achieved by the mycelium which is capable of living over in the dried tissue. The conclusion reached from the

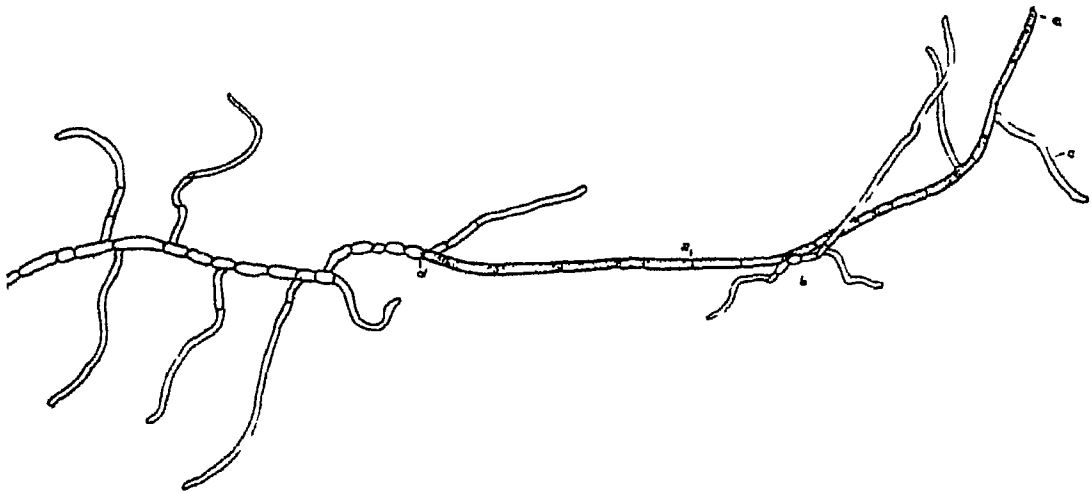


FIG. 3. Dried mycelium germinating.

TABLE 1—*Tabulated results of desiccation.*

Date	Drop number	Number of spores	Number of mycelial threads	Spores germinating after 21 hours	Mycelium germinating after 21 hours	Percentage of spores germinating	Percentage of mycelium germinating
Feb. 1	1	41	7	37	7	90	100
	2	18	10	17	9	94	90
	3	38	5	35	5	92	100
Feb. 10	1	28	4	12	4	43	100
	2	19	2	8	2	42	100
	3	35	6	20	5	57	83
Mar. 17	1	21	4	2	4	9	100
	2	35	6	3	5	8	83
	3	80	5	4	5	5	100
Apr. 10	1	20	5	0	4	none	80
	2	46	3	1	3	2	100
	3	18	8	0	7	none	87

study thus far is that the only function of the spores is that of dissemination during the growing season. After remaining dry for a few weeks the spores rapidly lose their vitality, while mycelium kept perfectly dry for nine months will readily germinate as shown in figure 3. This was concluded to be the fact only after many trials which yielded the same results. No samples of the diseased leaves now on hand have been kept for a longer period than nine months, so it is impossible to say for how long the mycelium will keep its viability.

The experiments regarding the viability of the spores and mycelium were conducted as follows. A culture of the organism growing on prune

TABLE 2—*Growth in millimeters at various temperatures.*

Tube number	Constant temperature ° C.	Diameter of growth at first	Diameter of growth after 4 days	Diameter of growth after 9 days	Diameter of growth after 15 days	Spore production after 15 days	Remarks
1	60	8	8	8	8	none	Organism killed
2	45	7	7	7	8	"	Growth very slight if any.
3	27	0	35	75	—	excellent	
4	21	0	24	55	80	good	
5	18	0	15	32	49	good	
6	11-15	4	6	10	15	fair	
7	10	4	5	7	10	poor	
8	3	4	4	4	4	few	The only evidence of growth was spores at center of colony
9	0	4	4	4	4	none	
10	-2	4	5	5	5	none	
11	-10	4	4	4	4	none	Medium frozen.

agar was evaporated to dryness in an incubator at a temperature of 33° C. When the culture seemed perfectly dry, three large hanging drops of spores and mycelium were made using prune agar as a medium. The following day by means of a mechanical stage the writer made a systematic count of spores and mycelial threads present in the drops, noting whether or not they were germinating. All groups where the mycelium was on top of or connected with the spores, all broken spores,

and all pieces of mycelium shorter than the length of a spore were disregarded. This was possible because of the large size of the spores. The above procedure was repeated ten days later with the same dried culture as a source of material, and again repeated in each of the two following months. The table above shows the results obtained. It will be noted that after ten days of drying a good percentage of the spores still retained their viability, but after three months practically none are capable of germination. However, in the case of the mycelial threads the percentage of germination after three months of drying is almost as high as it was after ten days. This demonstrates the fact that the mycelium will carry the disease over the winter while the spores are practically useless in this respect.

TEMPERATURE RELATIONS

The preceding table shows eleven different temperatures at which the growth of the organism was measured. The medium used was carrot agar. All of the cultures with the exceptions of numbers 3, 4 and 5, were kept at 21° C. for three days so that the colony had attained a diameter of 4 to 8 mm. before it was subjected to the temperatures indicated. Each was allowed to remain at a given temperature for fifteen days and the growth noted from time to time. It will be noted that the minimum temperature for growth was found to be about 3° C., the maximum about 45° C., and the optimum 30° C.

A MISLEADING SAPROPHYTE

A saprophytic *Alternaria*, which occurs on the diseased leaves with the *Macrosporium*, was introduced into the study because as a result of superficial examinations it has been considered and in some cases cited as being the cause of the blight. Through a series of inoculations it was proved beyond reasonable doubt that the only action of this organism is a saprophytic one. It does not grow in living tissue and does exceedingly well on dead leaves. The relation of this organism to temperature shows a much greater range than does the *Macrosporium*, demonstrating that it will withstand more unfavorable conditions than will the parasite.

A FUSARIUM WILT OF SPINACH

CHAS. W. HUNGERFORD

WITH FOUR FIGURES IN THE TEXT

During the summer of 1919 a disease of spinach was noted in several gardens in Moscow, Idaho, and in two other places in the state.¹ Since no report of the disease can be found in literature, a brief note of its occurrence is made at this time.

The first signs of the disease appeared when the plants were quite small. Here and there one was noted which was noticeably lighter colored than the rest and whose outer leaves were rolled inward at the margin and in more advanced stages were beginning to wither and die.



FIG. 1. Spinach plants growing in sterilized soil to which had been added a culture of *Fusarium spinaciae* when the seed was planted.

The leaves of the entire plant soon developed the same appearance and in about ten days these plants were entirely dead. Other plants soon developed this same condition and in about three or four weeks time, in the gardens where the disease was severe, there were only a very few plants which had not succumbed to the disease.

Upon pulling any of the plants in the advanced stages of the disease it was found that they came out of the ground very easily due to the

¹Dr. V. H. Young, Professor of Botany in the University of Idaho, first called the author's attention to the disease on spinach plants in his garden. Later it was also found in several other gardens in Moscow.

fact that the root system had been practically destroyed. Nearly all of the fibrous roots were rotted off and the main root itself was scarred and blackened. Upon splitting the crown and main root a marked vascular browning characteristic of certain wilt diseases was found.

When diseased roots were placed in a moist chamber a white fungous growth developed in spots over their surfaces. Upon examination this growth proved to be a species of *Fusarium*. The same fungus was isolated from the interior of diseased roots.

In the garden where the disease was first noted spinach had been planted in the same place two years in succession. Volunteer plants were numerous over this part of the garden during the spring of 1919 before the ground had been plowed. The disease was much more serious



FIG. 2 Spinach plants growing in sterilized soil as check.

in this garden than in any of the others where it was noted and although it is not known whether the disease was present the year before, it seems reasonable to suppose that it was and that the fungus overwintered either on these volunteer plants or in the soil.

Spinach seed of the variety known as Long Standing, which was the variety on which the disease was first noted, was planted in two flats of sterilized soil in the greenhouse. The soil in one of the flats was inoculated with a pure culture of the *Fusarium* which had been previously isolated from diseased spinach roots. This was done by mixing a culture of the *Fusarium* growing on steamed rice with the soil when the seed was planted. The other flat was left as a check. Very soon after the

plants began to emerge there was a very noticeable difference in the vigor of the plants in the two flats. Those in the inoculated flat were much slower in growth, lighter colored, and some of them soon developed the characteristic symptoms of the disease as it had been noted in the field.

Figure 1 shows plants grown in inoculated soil 24 days after planting. Figure 2 shows the uninoculated check at the same time. It will be noted that many of the plants in figure 1 were already dead and that none of them were as vigorous as those in figure 2. Figure 3 shows four



FIG. 3. Four spinach plants, on the left, taken from the check shown in figure 1 and four plants, on the right, grown in inoculated soil shown in figure 2.

healthy plants on the left taken at random from the check flat and four diseased plants, on the right, taken from the inoculated flat. The blackened areas on the surface of the roots of the diseased plants can be plainly seen.

The disease was found again in several gardens in 1920 and in 1921, but only a very few diseased plants were noted in any case. That the disease was so much more serious in 1919 may have been due to the fact that the season was exceedingly dry and the average soil temperatures were much higher than in 1920 when there was an unusually large amount of rainfall. During the summer of 1921 the disease was reported from

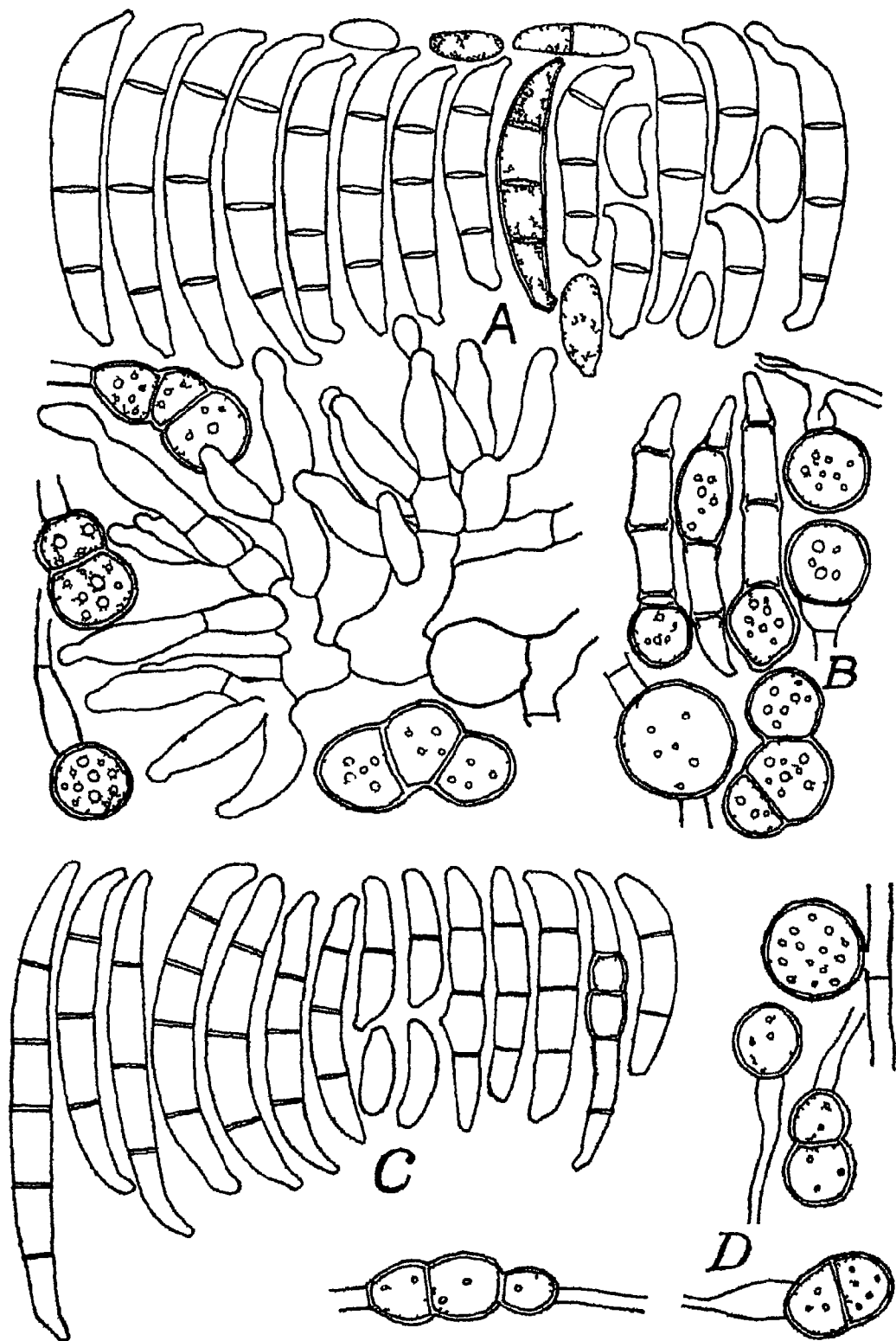


FIG 4 A Normal sporodochial conidia a conidiophore and chlamydospores from 27 day old test-tube culture on potato tuber plug with 5 cc of 5% of malt extract, B, 'old' sporodochial conidia with chlamydospores and mycelial chlamydospores from 8-months old test-tube culture on hard oat agar C, free conidia and D mycelial chlamydospores from 9 day old Petri dish potato agar Magnification 1000 times

Twin Falls County, which is in the irrigated region of southern Idaho. In 1922 it was reported from Lewiston and from Sandpoint. Specimens sent to the Experiment Station yielded what is apparently the same *Fusarium* that had previously been isolated.

As the *Fusarium* appeared to be different from any previously described a culture was sent for identification to Dr. C. D. Sherbakoff, Experiment Station, University of Tennessee, Knoxville, Tennessee. He considers it a new species and describes it as follows:

***Fusarium spinaciae* n. sp.** (Fig. 4).—Conidia, when borne in sporodochia, are typically broader toward, and usually abruptly constricted at, the apex; pedicellate; mostly 3-septate, 5.25×35 ($4.8-6.3 \times 27-43$) μ ; 1-septate, 4.8×20 ($4.4-5.0 \times 14-24$) μ ; 0-septate, 3.2×8 ($3.0-4.8 \times 6-11$) μ . Conidia, when borne singly on mycelium, are of a different type as shown in fig. 4, C. Chlamydospores mostly 0-septate, round and smooth, greatly varying in size, 11 (6–18) μ ; 1-septate common, many-celled are also numerous in certain media. Aerial mycelium from short to medium height, fine; no sclerotia, no pionnotes; plectenchyma is sometimes produced in form of small flakes; small, whitish sporodochia were observed on some media. Color of mycelium on various media without addition of sugar is white; with addition of maltose it is usually light lilac; on rice, when seven weeks old, color of aerial mycelium is, by Ridgeway's Standard, from light seashell pink to pale-purple drab; the line of the medium and mycelium from dark, deep vinaceous and pinkish vinaceous to purple vinaceous, with only a slight diffusion of the color into the medium. In the type of sporodochial macroconidia the fungus is very closely related to *F. redolens* Wr.; macroconidia when borne singly are of a type intermediate between *Fusaria* of the *Elegans* and *Martiella* sections; the cell walls are usually thicker and the contents more granular than in most of the *Fusaria* of *Elegans* section, thus somewhat approaching *Martiella* section; on certain media, such as soy bean agar, chlamydospores are very numerous and very large as compared with other species of *Elegans* section and, in this way, the fungus can be readily distinguished from other *Fusaria* closely related to this species.

Hab. On roots of spinach, *Spinacia oleracea* Mill., Idaho, U. S. A.

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OCCURRENCE OF BUNT IN RYE¹

E. F. GAINES AND F. J. STEVENSON

WITH TWO FIGURES IN THE TEXT

Bunt or stinking smut, *Tilletia tritici* (Bjerk.) Wint., is not confined to wheat, *Triticum* sp. but also attacks rye, *Secale cereale* L. In the literature referring to this parasitic fungus, it has been taken for granted, apparently, that it has but one host. Special mention is made of this fact by Sutton (3) and Humphrey and Potter (2), who state that bunt is limited to wheat as a host.

In the fall of 1921, one plant of common rye, one F₂ plant of a rye wheat hybrid, and two F₂ plants of a wheat-rye hybrid were found affected with smut, of which the fetid odor, and the size, shape, and roughened surface of the spores left no doubt that it was *T. tritici*. Figure 1 shows two bunted heads in comparison with a normal head of rye.

These hybrids were made at the Washington Agricultural Experiment Station with Rosen rye as one of the parents. As reported elsewhere (1), Rosen has been found peculiarly congenial in crossings with wheat, for fertile hybrids have been obtained when this rye was used as the female parent. Three such crosses have been made, Rosen × Hybrid 128, Rosen × Jones Fife, and Rosen × Jenkin, but every plant of the F₁, the F₂, and the F₃ generations has resembled rye, or the female parent, in its botanical characters. The wheat-rye hybrid, Turkey × Rosen, also resembled the female parent in the F₁, the F₂, and the F₃ generations, although it was different in many respects from Turkey. (Such hybrids in which the species of the pollen parent is not recovered in succeeding generations are rare, few ever having been cited, the best known in crosses with wheat being *Aegilops* × *Triticum vulgare*.) Bunted plants found in these hybrids, especially in Rosen × Hybrid 128, which was like rye in all its taxonomic characters, were thought to show genetic relationship with the wheat parent. To find bunt on rye, however, could have but one interpretation, namely, that rye is a host of *Tilletia tritici*.

To make a further test of this and to determine to what extent bunt infection of rye could be induced by artificial means, nine winter ryes

¹ This paper presents the results of investigations conducted cooperatively by the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Washington Agricultural Experiment Station.

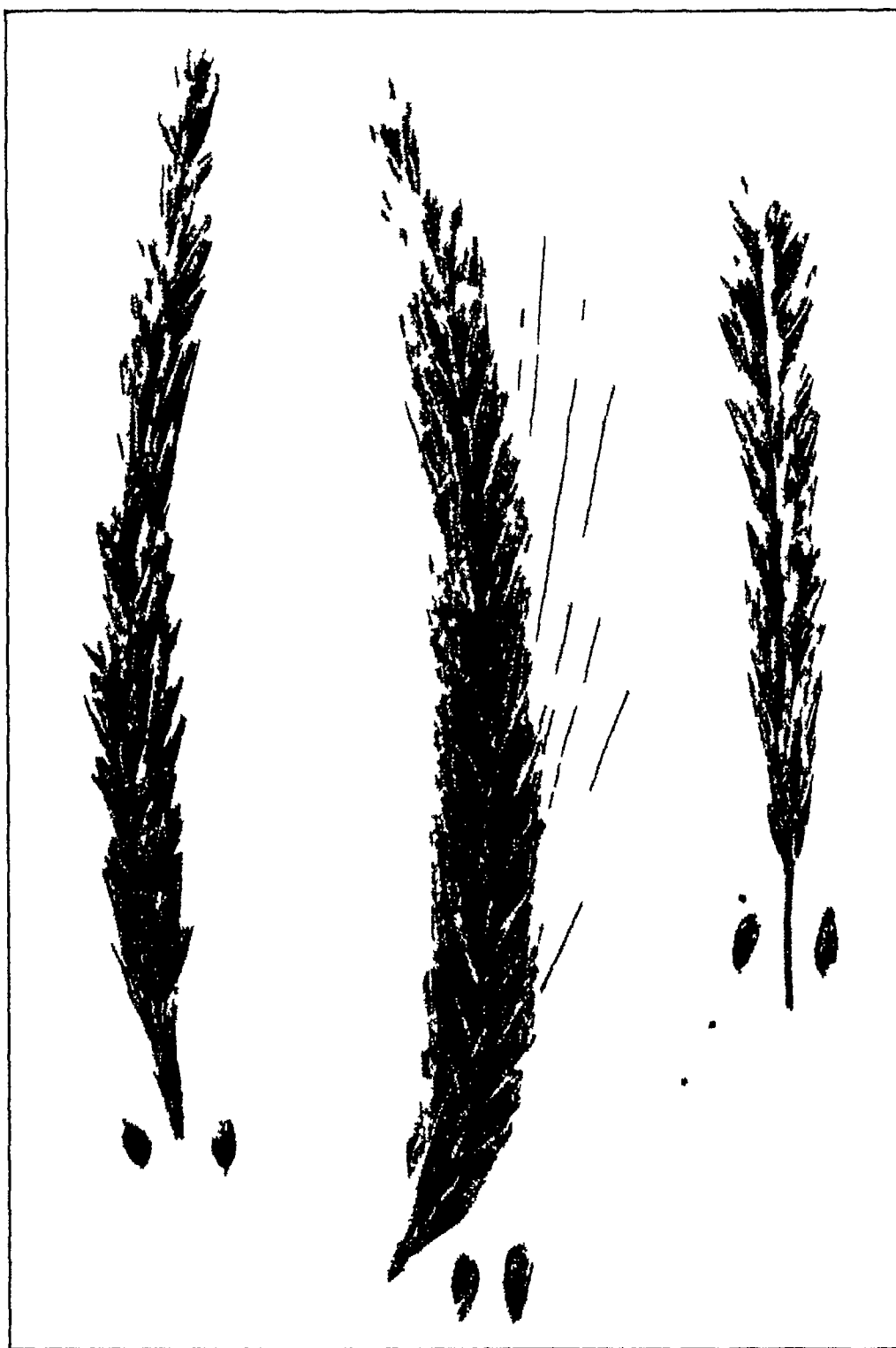


FIG 1 First heads infected with bunt ever found on rye at the Washington Experiment Station Pullman, Washington Normal head in center
Row 9001 A Cereal Nursery, August 6 1921 ($\times 1$)

and the F_2 generation of each of the four hybrids referred to above were sown under conditions favoring maximum infection. The seeds were blackened with spores of bunt obtained from smutted heads of several varieties of wheat and sown October 8, 1921, in a moist seed bed, thus insuring a combination of optimum conditions for infection. Fourteen spring ryes were treated in the same manner and sown April 29, 1922. The results of the 1922 harvest show that four out of the nine varieties of winter rye were made to act as hosts for bunt, although these four were relatively resistant to its attacks, while the other five apparently were immune.

TABLE 1—*Number of bunted and normal plants in rye and rye-wheat hybrids in comparison with those in wheat and wheat-rye hybrids*

Crop	Variety	Wash. No.	Sown in	No. of plants	
				Normal	Bunted
Rye	Utah	1741	autumn	55	5
"	Common	1738	"	61	1
"	German	1742	"	46	1
"	Yellow	995	"	35	0
"	Green	997	"	36	0
" (Parent)	Rosen	1940	"	100	0
Rye $\times$ Rye F_2	Spring $\times$ Minn. No. 2	999 $\times$ 991	"	14	2
" "	" " "	999 $\times$ 991	"	23	0
" "	" " "	999 $\times$ 991	spring	27	0
"	Spring	998	"	75	0
"	"	999	"	11	0
"	Mexican (S. P. I. 26102)	992	"	46	0
"	Dakold (C. I. 175)	996	"	57	0
"	Abbruzzes	994	"	57	0
"	C. I. 169	993	"	35	0
"	German	1742	"	38	0
"	Common	1738	"	60	0
"	Minn. No. 2	991	"	38	0
"	Yellow	995	"	46	0
"	A. Beardless	1086	"	48	1
"	Common	2349	"	17	0
"	Utah	1753	"	50	0
"	Rosen	1940	"	2	0
Rye $\times$ Wheat F_2	Rosen $\times$ Hybrid 128	1940 $\times$ 592	autumn	2008	68
" "	Roxen $\times$ Jones Fife	1940 $\times$ 371	"	503	3
" "	Rosen $\times$ Jenkin	1940 $\times$ 526	"	421	2
Wheat (Parent)	Turkey	326	"	42	25
" "	Hybrid 128	592	"	8	47
" "	Jenkin	526	"	3	10
" "	Jones Fife	371	"	3	24
Wheat $\times$ Rye F_2	Turkey $\times$ Rosen	326 $\times$ 1940	"	27	74

Table 1 gives the results of the infection experiments of 1922 on the winter and spring ryes and on the various rye-wheat and wheat-rye hybrids and their parents.

All plants that produced any smut balls at all were listed as bunted although some of them contained normal as well as bunted heads. The



FIG. 2. A bunted and normal head of Yellow Rye (left) and of Rosen (right), found in the field plots, Washington Experiment Station, Pullman, Washington, in 1922. ($\times \frac{1}{2}$.)

F₂ of Turkey wheat $\times$ Rosen rye consisted of ten rows which showed infection with bunt ranging from 25 to 100 per cent. Thirty-eight of the seventy F₂ rows of the Rosen $\times$ Hybrid 128 family produced one or more affected plants while one row showed an infection of 10 per cent of bunted plants. Thirty-nine F₂ rows of Rosen rye $\times$ Jones Fife wheat were smut-free, but three others produced a total of eight bunted heads. In the other rye-wheat cross, Rosen $\times$ Jenkin, two of the 14 F₂ rows produced one infected plant each. The parents of these four crosses were sown as controls with the following results: Rosen rye was bunt

free, Turkey had 11 per cent, Hybrid 128 had 84 per cent, Jones Fife had 81 per cent, and Jenkin had 85 per cent of bunted heads.

The spring ryes were all bunt-free with the exception of Beardless which produced four bunted heads on one plant in a rod row containing 49 plants. Aside from these tests, three bunted plants were found in the field plots; one in a plot of Yellow rye and two in a similar plot of Rosen, due presumably to soil infestation. Types of each variety are seen in figure 2.

Rosen is supposed to be a true winter variety. The two plants which headed no doubt were mixtures or the result of field hybridization the previous year. One of the bunted Rosen plants produced a normal head which contained green and yellow seeds in the ratio of three to one, indicating that it came from a field hybrid seed, presumably wind pollinated the year before from the adjoining plot of Yellow rye. Thus it seems likely that the two bunted plants found in the Rosen plot were not pure Rosen, but hybrids with other ryes, as the nursery rows of pure Rosen could not be induced to produce any bunt at all.

The question naturally arises as to whether there may have appeared a new specialized form of bunt capable of attacking rye, and which might be more virulent on wheat than the common bunt. To test this point, three susceptible wheat varieties, Baart, Jenkin, and Pacific Bluestem, were chosen and the seed divided into three lots. The first lot was blackened with spores from the bunted rye head of the previous season, the second with spores from a bunted rye-like plant of the F_1 generation of Rosen rye $\times$ Hybrid 128 wheat, and the third with spores from a wheat-like plant found in the F_2 generation of Turkey wheat $\times$ Rosen rye.

A few bunted heads were produced in every case, but there were not as many as would have been expected if the seed had been inoculated with spores from the normal host. The results are shown in table 2.

TABLE 2—*Infection of susceptible spring wheats with bunt from spores obtained from different hosts.*

Host of Inoculum	Date seed was sown	Number of plants obtained					
		Baart		Jenkin		Pacific Bluestem	
		Normal	Bunted	Normal	Bunted	Normal	Bunted
Rye	May 4	60	7	50	6	57	6
Rye-Wheat	May 4	60	2	56	5	66	5
Wheat-rye	May 4	62	0	18	42	67	0
Wheat	April 29	10	47	8	79	12	72

The results obtained from seed inoculated with spores from wheat are not strictly comparable with the others, as the seeds were sown on April 29, while the others were sown on May 4, five days later. Earlier sowings usually produce more bunt, as wheat will germinate in a wider temperature range than will the smut spores.

Three varieties of spring rye also were inoculated with spores from these same four hosts, but no bunt appeared. Thus it appears that *Tilletia tritici* may pass one generation on rye as host, and still attack wheat feebly, but it is not shown to produce a second generation on rye.

From the description of *Tilletia secalis* (Cda.) Kuhn, given in Saccardo's Sylloge Fungorum, it seems possible that *T. tritici* and *T. secalis* are in reality the same fungus growing on different hosts. The rarity of "*T. secalis*" is in keeping with the great resistance of the rye varieties to *T. tritici* as determined in the above experiments.

The possibility of using bunt as an index of genetic relationships between wheat and rye in the rye-wheat hybrids is at once apparent. These hybrids, after the first generation, have all resembled the female parent to such an extent that they might be mistaken for pure-line ryes. This is true of the third generation families of Rosen $\times$ Hybrid 128, but the fact that they were quite susceptible to bunt as compared with the Rosen rye parent, which was bunt free in the control rows, indicates that they were true hybrids. Susceptibility to bunt, used as such an index, makes the work with hybrids of this nature much more significant than was formerly supposed, as it shows that something more than a stimulation has gone into the egg in the fertilization process. At least part of the chromatin material of the wheat pollen must be present, and functional in many of the F₁ segregates.

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PULLMAN, WASHINGTON.

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STUDIES ON THE SO-CALLED BLACK HEART DISEASE OF THE APRICOT

HELEN CZARNECKI¹

WITH PLATE XI AND FOUR FIGURES IN THE TEXT

A disease of the apricot manifesting itself by a sudden wilting of the foliage and discoloration of the wood has been under observation for several years.

In the spring of 1916, the writer became interested in the general group of diseases commonly termed "sour sap." During the months of March and April numerous cases of sour sap occurring on various stone fruits were observed, this being mainly a bark and cambium trouble, while in June a disease also included in this group, which affected the wood tissue only, was observed. These two marked differences led the writer to believe that the latter was a distinct type of disease and further study was made of this particular form.

DISTRIBUTION

The disease has been observed on the apricot in Alameda, Santa Clara, San Benito and Ventura Counties, and on the almond in Merced County. It has also been reported as occurring to a slight extent in Kings and Orange Counties. The disease is most commonly found in three to six-year-old trees which are heavily irrigated during the summer and inter-cropped with tomatoes. (Fig. 1.) It is not uncommon, however, to find the trouble both in younger and older trees. In several nurseries in Santa Clara County apricot seedlings and apricot and Myrobalan seedlings budded with apricot a month or so previous showed the disease. The distribution in the nursery was confined to certain blocks, while often adjoining blocks were healthy.

Many orchards of Alameda and Santa Clara Counties were visited. In Alameda County, in the young plantings of Centerville and Decoto apricot districts, the disease was observed in a large number of orchards; usually most of the trees were affected, although it was not uncommon to find only one or two affected trees to an orchard. A similar condition was found in the apricot orchards of Santa Clara County. For several

¹ This paper was prepared in 1917 by Miss Czarnecki, while a graduate student in Plant Pathology at the University of California. Publication has been unavoidably delayed until the present time. RALPH E. SMITH.

years Mr. E. L. Morris, formerly Horticultural Commissioner of Santa Clara County, had observed the disease in a large number of the orchards of his district. Mr. A. A. Brock, formerly Horticultural Commissioner of Ventura County, has reported that the disease has been observed in many of the younger apricot orchards throughout Ventura County. Mr. R. K. Bishop, formerly Horticultural Commissioner of Orange County, has observed it to a limited degree in Orange County. Mr. F. K. Howard reported that the disease had been quite common in Kings County for many years



FIG. 1. Young apricot tree, typically affected with black heart.

THE DISEASE

The disease usually begins to show early in June. The leaves at the tip of one or more branches of a tree wilt and the wilting progresses rapidly downward until the entire branch is affected. The wilted leaves turn yellow and finally fall (Fig. 1). Previous to this time, so far as the writer has observed, no external symptoms of a diseased condition

can be detected. The foliage and fruit develop normally up to the time that wilting commences. Often those branches which become partly or wholly defoliated produce a pale yellow new growth later in the season. This wilting of one or more branches may continue until the dormant season. Upon cutting into a wilted branch the wood shows a brown to black stain (Pl. XI, A). The discoloration is often confined to one or

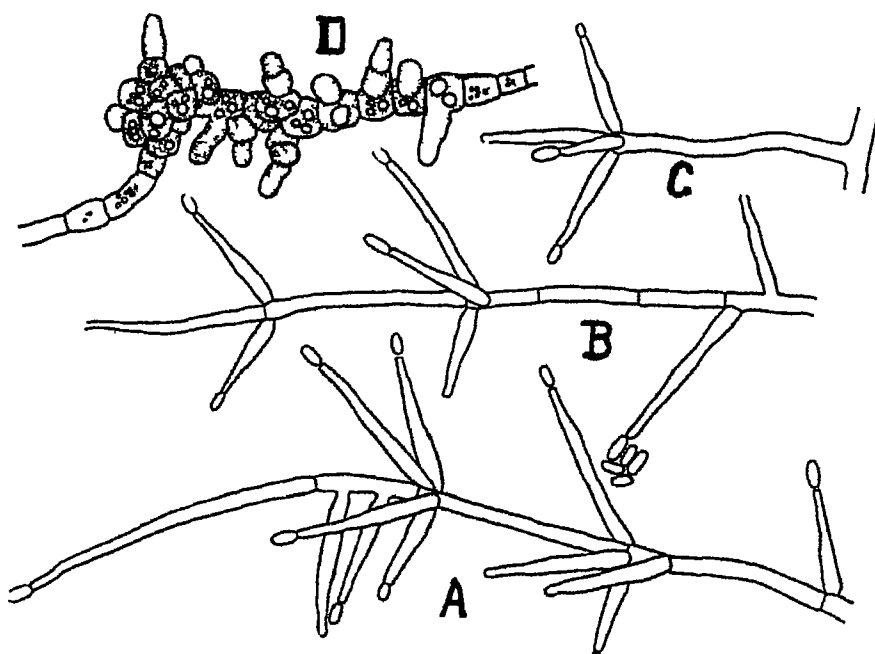


FIG. 2. *Verticillium* on standard nutrient agar. A, B, C—Verticillately branched conidiophores bearing single acrogenous conidia from 6-day-old nutrient agar culture. D. Aggregation of gemmae forming sclerotial mass, also from 6-day-old nutrient agar culture.

more annual rings and so, on cross-sectioning an affected branch, concentric black rings are observed. This discoloration was traced from affected branches into the main trunk and in no case was it possible to cut below the discolored area. However, the discoloration was less marked in the lower part of the trunk. Several dozen trees were examined, but in every case the discoloration was traced into the main trunk to the crown. The discoloration often extended into the main root, a few inches from the crown, but the laterals showed no darkening

of the tissues. These observations were made on two-year-old trees when the disease was fully developed for the season. These trees had the disease the previous season. Several horticultural commissioners, however, have reported that in cutting out wilted branches as soon as they appear, they have been able to cut below discolored wood. The sudden wilting of the branches is the mechanical effect of the formation of gum in the xylem vessels and the cutting off of the water supply of the tree.

In the literature of plant diseases, the only record which we have found of the occurrence of any similar disease is a disease of the plum in Georgia, called plum wilt. According to Higgins,¹ plum wilt is a destructive disease of the Japanese and hybrid varieties of plum in Georgia, North Carolina and Alabama. It manifests itself very similarly and is caused by a fungus of the genus *Lasioidiplodia*.

ISOLATION OF FUNGUS

On June 5, 1916, material was obtained from an affected apricot orchard at West San Jose, Santa Clara County. Frozen sections were made of affected branches, and the xylem vessels were seen to be filled with mycelium (Pl. XI, D). On June 7, cultures were made from this material. The branches were scrubbed and soaked in mercuric chloride (1-1000 in distilled water) for 15 minutes, rinsed in sterile distilled water, the outer bark peeled off with a scalpel sterilized in the flame and sections cut off the underlying tissue. Plantings were made on Shear's corn meal agar, standard nutrient agar, and in standard nutrient broth. Six cultures were made with each of the media.

On June 10, all six cultures on the corn meal agar showed an embedded fungus growth extending 1 to 3 mm. from the chip of wood. Five of the cultures on standard nutrient agar and four of the broth tubes showed a similar growth. After one month all six of the bouillon tubes showed a white pellicle on the surface and in the mycelium of the pellicle an abundance of small black sclerotial masses were produced. A moderate growth extended from the piece of twig at the bottom of the tube and an abundance of spores were produced which germinated in the liquid and produced a very characteristic flaky growth.

On June 14, eleven out of the twelve petri dish cultures which showed growth were transferred to nutrient agar slants. The original cultures were also kept and, with the exception of *Penicillium* contaminations, showed a pure culture of the fungus (Plate XI. C).

¹ Higgins, B. B., Plum wilt, Georgia Agric. Exp. Sta. Bull. 118, 29 p. *illus.* 1916.

Isolations were made from material collected at Berkeley, Oakland, Centerville and Decoto, Alameda County; Le Grand, Merced County; San Jose and Edenvale, Santa Clara County. One hundred and eighty two isolations, varying in number from 3 to 42 from each locality, yielded fungus growth in 137 instances.

All of the above isolations were made from 3 to 6 year-old apricot trees, with the exception of the material from Le Grand, Merced County, which were from two-year-old almond trees. The fungus was also isolated from imported *Myrobolan* seedlings, budded with apricot a month or so previous and also from seedling apricots budded with apricot.

HISTOLOGIC STUDIES OF AFFECTED TISSUE

A cross section of a badly discolored twig shows the cells of the bark and wood tissue to be filled with a mass of brown gum, especially the cells

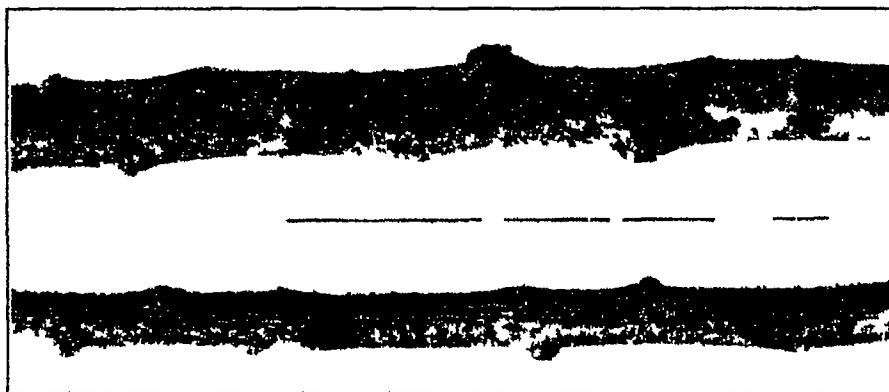


FIG. 3. Fruiting bodies on apical twigs affected with black heart.

of the medullary rays (Pl. XI, B). Either in frozen or paraffin sections, the fungus can readily be seen in the xylem vessels. In sectioning the tips of young growing branches 3 mm. in diameter, which show discoloration, many of the xylem cells show an abundance of the fungus mycelium, and the surrounding cells are filled with a brown, gummy, granular substance.

THE FUNGUS

The mycelium is uniform, septate, branched, 1.8–2.7 μ broad. Aerial upright verticillately branched conidiophores, 35–200 μ in length bearing conidia $6.75\text{--}11.25 \times 2.25\text{--}6.75 \mu$ at the apex of the branchlets, (Fig. 2), are produced in nutrient agar petri dish cultures after 6 days.

The spores germinate in dilute prune juice, producing tubes 3–45 μ

in length, 5-6.5 μ wide from either or both ends of the spore after 24 hours. In three days irregularly branched conidiophores begin to pinch off conidia. Often a solitary branch arising from the main mycelium produces spores from the tip. In 4-5 days the main mycelium divides and an abundance of gemmae are produced (Fig. 2, D).

CULTURAL STUDY OF THE FUNGUS

The cultural work up to the present time indicates that the fungus belongs to the genus *Verticillium* and is apparently a new species, as no previous record of a *Verticillium* of a true parasitic nature inhabiting



FIG. 4. Two-month-old culture of *Verticillium* on sweet potato, showing the compact aerial growth.

the sound wood tissue of the apricot, almond or any other fruit tree has been found.

In culture, the fungus produced a similar growth on all the media tried, which included standard nutrient broth, standard nutrient agar, potato, prune and corn agar, steamed rice, oatmeal, string bean pods and sweet potatoes. In the liquid nutrient broth an abundance of spores is produced from the piece of transplanted fungus. The spores germinate and produce a very characteristic flaky growth. In 7 to 8 days a pellicle is formed on the surface and in the mycelium of the

pellicle sclerotial masses are formed, which consist of an aggregation of dark vacuolate gemmae. In the agar cultures, which included standard nutrient, prune, corn, potato and oat agar, an even, embedded growth with a scant production of verticillately branched aerial conidiophores was produced within six days. After 10 days an abundance of the black aggregate masses of gemmae was produced irregularly throughout the embedded growth and on the surface of the medium. In media rich in starch, such as steamed rice, oatmeal, and sweet potato, an abundant compact aerial growth was produced (Fig. 4). As the cultures became older, black sclerotial masses formed in the mycelium and after several months the white mycelium flattened down and the sclerotial formation continued until a black crust was formed on the surface of the medium. In old cultures secondary white aerial tufts were produced.

TABLE 2—*The rate of growth and development of the fungus at the various temperatures.*

Culture No.	Temperature Degrees F.	Age of culture	Development
1-6 inc.	55	24 hrs.	Growth—1 mm.
7-12 "	64	24 "	Growth—2 mm.
13-18 "	73	24 "	Growth—2 mm. Production and germination of spores.
1-6 "	57	48 "	No increase in growth.
7-12 "	68	48 "	Growth 3 mm. Production and germination of spores.
13-18 "	75	48 "	Growth 5 mm. Increased pro- duction and germination of spores.
1-6 "	57	3 days	Growth 3 mm. Scant produc- tion and germination of spores
7-12 "	68	3 "	No increase in growth.
13-18 "	68	3 "	No increase in growth.
1-6 "	50	5 "	No increase in growth.
2-12 "	68	5 "	Growth 8 mm.
13-15 "	77	5 "	Sclerotia formed on side of flask.
16-18 "	99	5 "	No increase in growth.
1-6 "	50	10 "	No increase in growth.
7-12 "	68	10 "	Sclerotia formed on side of flask.
13-15 "	77	10 "	Pellicle formed on surface and an abundance of sclerotia.
16-18 "	98	10 "	No increase in growth.

FORMATION OF SCLEROTIA

In single spore dilute prune drop cultures, portions of the mycelium divide into short cells or gemmae. These cells broaden and become dark in color, and secondary gemmae are produced. In single spore cultures on standard nutrient agar, the gemmae multiplied until black sclerotial masses 50 to 250 μ in diameter were produced.

TEMPERATURE RELATIONS

After 10 days cultures in standard nutrient broth kept at 95° to 99° F. produced a scant growth in the liquid; at 68 to 77° F. a luxuriant growth in the liquid; a heavy pellicle on the surface and abundant sclerotia; at 64 to 68° F. a moderate growth in the liquid and a few sclerotia; at 50 to 57° F. a scant growth in the liquid.

INOCULATIONS

One set of inoculations was made in June, 1916, on a five-year-old seedling apricot. Twenty four one-year-old branches were selected, thoroughly washed with corrosive sublimate (1: 1000 in distilled water) and a flap was cut through the bark into the wood of each twig. A piece of fungus was placed under the flap of 12 twigs and the remaining 12 left as controls. All were tied with thread and covered with paraffin paper.

After one month, cultures were made from an inoculated branch as follows:

1-2 inc. 2'' from slit—wood discolored—contaminated by bacteria.

2-4 inc. 4'' from slit—wood discolored—positive growth of *Verticillium*.

5-6 inc. 6'' from slit—wood discolored—positive growth of *Verticillium*.

7-8 inc. 8'' from slit—no discoloration—positive growth of *Verticillium*.

9-10 inc. 10'' from slit—no discoloration—no growth.

11-12 inc. 12'' from slit—no discoloration—no growth.

These results, together with the isolations of the fungus from black heart material from various sources as stated on page 8 corroborate the constant association of the fungus with this disease.

SOME LIGHT ON THE NATURAL MODE OF SPORE FORMATION

In January, 1916, it was observed that many of the affected trees showed numerous dead twigs, on the surface of which an abundance of black fruiting bodies 0.5 to 1 mm. in diameter had developed (Fig. 3).

Freehand and paraffin sections showed these bodies to be an aggregation of gemmae into a hard mass, a body similar if not identical with the sclerotial mass produced by the *Verticillium* in culture. No definite stroma or spore cavity was observed. These bodies were often covered with a greenish growth of *Cladosporium*. Every attempt to culture the fungus by sterilizing the surface of the twigs with corrosive sublimate and then planting the fruiting bodies on standard nutrient agar failed. An abundant growth of the *Cladosporium* and other saprophytes was obtained, but nothing suggestive of a *Verticillium*. Blackheart twigs were placed in water and kept in the open for several weeks and after a rain of several days, fruiting bodies which appeared free from the *Cladosporium* growth were found. From the gemmae on the surface of these black sclerotial masses, short solitary conidiophores, bearing conidia at the apex, were produced. Poured plates were made from these spores and a mixed growth of the *Cladosporium* and the typical *Verticillium* was obtained.

On June 6, 1916, small twigs showing typical black heart discoloration were cut into 4 inch lengths, soaked in mercuric chloride (1:1000 in distilled water) for 15 minutes, rinsed in sterile distilled water, the outer bark peeled off with a scalpel sterilized in the flame, and each placed in a test tube containing 5 cc. of sterilized distilled water. When the twigs were examined March 12, 1917, the surface was covered with sclerotial masses similar to those on the affected twigs obtained from the orchards.

PROBABLE CONTROL

The probable method of control will be tree surgery, by immediately cutting out wilted branches if it proves possible to cut below affected areas, or else by removing the entire tree in the fall, in order to prevent spore infection the following year from the fruiting bodies on the dead twigs of affected branches.



BLACK HEART OF APRICOT

- A Longitudinal section of affected branch showing characteristic discoloration.
 B Cross section of small twig showing bark, wood and pith cells clogged with gum.
 C Fungus after one month's growth on standard nutrient agar in chip of wood from upright branch affected with black heart.
 D Longitudinal section of small twig showing mycelium in the xylem vessels and lignified gummy substance in the cells.

FUSICOCOCCUM CANKER ON APPLE

CHARLES CHUPP AND GRACE L. CLAPP

WITH PLATE XII

Young Duchess of Oldenburg apple trees were sent in February 1921 from a storage cellar at Newark, N. Y., to the State College of Agriculture for examination. Accompanying the specimens was a letter stating that about a hundred nursery trees of this variety were affected with black cankers at or near the bud union, and occasionally farther up the stem near the branches. None of the varieties making up the remainder of the stored stock were affected.

The specimens when examined showed black cankered areas 4 to 6 inches long, partly encircling the scion and frequently on the stock just below the graft insertion. The cankers were smooth externally, not depressed, and brown under the bark, the discoloration extending entirely through the sap wood (Pl. XII, fig. 5). Superficially the symptoms were somewhat the same as those caused by *Bacillus amylovorus*, particularly since no fungous fruit-bodies were present. Subsequently, it was learned that the causal organism seldom fruits until the host has been killed.

In cross section the discolored area is found to be filled with branched, septate, hyaline mycelium 1 to 2 μ in diameter. It is both inter- and intracellular. Minute stromata form immediately below the periderm of the host. These increase rapidly in size and soon show enclosed conidiophore layers near their centres. By this time the periderm has been pushed outward, and finally breaks, due to the mechanical pressure applied to it, thus permitting a portion of the stroma to protrude (Pl. XII, fig. 7). This opening or hole is at first a narrow slit, but gradually becomes circular. The protruding stromata are usually grouped very closely together and are quite small as compared with the pycnidia of a species of *Cytospora* which is rather common on weakened apple twigs, and which occasionally may be found on the same limb with the fruit-bodies described above (Pl. XII, fig. 6).

Isolations were made by planting in potato agar pieces of tissue taken at the line of demarcation between the healthy and diseased areas. Pure cultures of a rather slow growing fungus were easily obtained. Various types of media such as apple agar, cooked apple, sterilized apple twigs, oat agar and potato agar were used in the culture work.

The organism grew equally well on any of the media, so that for most of the later work, potato agar alone was employed. On this medium as well as on other agar concoctions, the fungus grows appressed to the surface. At first the mycelium is white but gradually changes to *courge green* (Ridgeway). In old cultures it is almost totally black with patches of white aerial mycelium. The growth is rather slow, taking 7 to 10 days to spread from the centre to the margin of a nine centimeter petri dish when the temperature is at the optimum.

After two to four months the surface of the cultures is marked with numerous tussock-like stromata (Pl. XII, fig. 4) in which are found the spore bearing cavities. The number of these cavities in each stroma varies from one to several. They are very irregular in outline, and differ much in size. The principal cavity ordinarily is ellipsoidal with one or two partly separated chambers branching from the main loculus. The cavities are lined with thin long, straight conidiophores arising from cells of the pseudoparenchymatous tissue.

Arising from similar cells and thickly interspersed among the conidiophores are thin, long, hyaline, variously curved, unicellular bodies, which in another species have been named scolecospores by Shear (7) and are generally referred to by European writers as pseudo-paraphyses (Pl. XII fig. 8). The latter term is probably the more desirable since they have never been induced to germinate. Furthermore, the prefix to the term would differentiate them from the true paraphyses present in ascomycetous fruit-bodies. The pseudo-paraphyses are deciduous, and not only are found emerging with the conidia, but are present in abundance after the conidia have all been discharged, and may then be seen exuding in a grayish slimy mass.

The conidia are hyaline, one-celled, and cylindrical with tapering ends. They show two very pronounced oil-droplets, which in darkly stained preparations have the appearance of cross walls. Singly they appear hyaline; in mass they are yellowish and discharged in long orange-colored tendrils. They germinate readily at room temperature in 24 hours.

The fungus in question is a *Fusicoccum*. Several of the species of this genus have been described as occurring on dead or living parts of fruit trees. Ellis and Everhart (2, p. 287) describe *F. persicae* on dead limbs of peach trees. Potebnia (4, p. 15) lists *F. pruni* on plum. Reddick (5) proved that *F. viticola* was the cause of the dead-arm disease of grapes, while three species have been found on apple, namely, *F. complanatum* Delacr. (1), *F. malorum* Oudem. (3, p. 529) and *F. microsporium*

Potebnia (4). This last species, as described by Potebnia, has a stroma like that of *F. complanatum*, but the spores are smaller than those of the latter fungus. Saccardo points out that Potebnia was working with immature specimens, and that during the following June spores 10 μ in length were produced. He, therefore, makes *F. microsporium* synonymous with *F. complanatum*. Other species have been found on widely different hosts.

Roberts (8) has described a fungus on apple, and following Diedicke's classification (9), has named it *Phomopsis mali*. As far as spore measurements are concerned it compares closely with the *Fusicoccum*. In other respects it is quite distinct. According to Roberts, "The globose rather large pycnidia of the fungus develop singly or in a raised stroma from one-half to one cm. across." The *Fusicoccum* pycnidium, on the other hand, is comparatively small, elliptical rather than globose, and no stromae have been found which measured even a thousand microns across. Furthermore, the cankers are perfectly smooth so that they never suggest a "rough-bark" condition, and leaf inoculations have not proved successful as were those reported by Roberts for *Phomopsis* (10).

As far as the gross morphological characters are concerned, the fungus on the Duchess nursery stock differs from all the described species with the possible exceptions of *F. viticola* Reddick and *F. malorum* Oudemans. For instance, in *F. complanatum*, of which type specimens from Delacroix's collections of 1890 and 1908 were kindly sent to the writers by Dr. E. Foëx of France, the spores differ from those of the fungus in question both in width and length, and the pseudo-paraphyses are unlike, being 4 to 5 μ in width, very long, branched, and multiseptate.

The description of *F. viticola* Reddick, on the other hand, fits the fungus very closely, and the possibility presents itself of the dead-arm organism also causing a canker on apple. Two different collections of dead-arm were obtained from the Chautauqua grape district and isolations made. The cultures on potato agar at first resemble very closely those that were obtained from apple, that is, the mycelium is closely appressed to the agar and, snowy white, but it grows much more slowly (Pl. XII, fig. 9) and does not turn to course green or black. In addition when apple twigs on growing trees are inoculated, no canker results, although inoculations made with the apple fungus on the same trees give typical cankers in from three to four weeks. The inoculations are made by first washing the twig with a corrosive sublimate solution, and rinsing with sterilized water. A leaf petiole is then removed close to the twig with a flamed scalpel, a small amount of mycelium culture

applied, after which the lesion is wrapped in cotton which has been rinsed in sterilized water. The cotton is protected by lead foil wrapped over it. It has been found that the apple fungus will not produce infection unless the host is first injured. When the inoculum is applied over a cut leaf petiole, the results apparently are surer than when inoculations are made directly through the bark. In a similar manner inoculations were made on grape. The apple fungus did not cause dead-arm, while definite lesions were produced by *F. viticola*.

From the results of these reciprocal inoculations and reisolations together with the differences in cultural characters, such as color and manner of growth, it seems safe to conclude that the organisms on apple and on grape are two distinct species.

It is not so easy to prove that the apple fungus is different from species named by Oudemans as *Fusicoccum malorum*, and which he described as follows:

"Stromata wartlike, black, erumpent, of various forms, with very uneven surface, irregularly chambered, the chambers lined with straight, rather long sporophores. Spores spindle-shaped, hyaline, unicellular, smaller at each end, $7-9\ \mu \times 2-3\ \mu$."

Nothing at all is said regarding pseudo-paraphyses which are usually the most striking parts of the pycnidium. Furthermore, Oudemans' fungus was found on the apple fruit and not on the branches. This may be an important difference inasmuch as the *Fusicoccum* found on the nursery stock probably never causes a rot or spotting of the fruit under natural out-door conditions. It is true that the organism will cause infection when artificial inoculations are made, but the progress of decay is so slow that even under optimum temperature and humidity it takes six weeks to get any appreciable amount of infection, and six months to decay the whole fruit, or to obtain spores for dissemination.

The description of *F. malorum* being so meagre, a request for type material was made. Dr. J. C. Schoute, director of the Gröningen Botanical Laboratory of the State University, Holland, kindly searched through the herbarium left by Dr. Oudemans, but could find no material under that name, or under any possible synonymous name. The lack of type material makes it impossible ever to compare the two organisms. Consequently, it is considered better to place the apple canker fungus under a new species and describe it as *Fusicoccum pyrorum* n. sp.

***Fusicoccum pyrorum* n. sp.**

Stroma very pronounced, at first covered by the periderm of the host, later erumpent, enclosing one to several irregular plurilocular pycnidia,

horizontally ellipsoidal, from 300 to 500 $\mu \times$ 450 to 900 μ . The cavities are lined with narrowly lanceolate, hyaline conidiophores approximately 15 $\mu \times$ 1 μ . Interspersed among the conidiophores are thin, long, hyaline, variously curved, deciduous pseudo-paraphyses, 0.5 to 1.8 μ in width and 15 to 30 μ in length, averaging $1.2 \times 23.4 \mu$. Conidia are one celled, cylindrical, bi-guttulate with tapering ends, and discharged in yellow tendrils. The conidia vary in length from 6 to 10 μ and in width from 1.5 μ to 3.5 μ averaging $2.5 \times 7.5 \mu$.

Produces cankers on living branches and trunks of *Pyrus malus* L.

The ecological relationships of the parasite were not studied, but incidentally a few observations were made. At first the cultures were placed in an incubator registering 27° C. At this high temperature, the mycelium grew very slowly, and fruit bodies were lacking entirely. When the temperature was reduced to 25° C. the mycelium grew luxuriantly, still no pycnidia were formed. All the cultures, however, when removed from the incubator and held at room temperature (18 to 21° C.) produced pycnidia abundantly.

The fungus is found on trees that first have been injured or weakened from some other cause. The Duchess of Oldenburg trees, on which the organism was first discovered had been placed in storage, and evidently were not in as vigorous condition as was the other stock in the same storage house. The same disease was found in Wayne County, N. Y., during the summer of 1922 on young Twenty Ounce trees that had been damaged by low temperatures during the previous winter. Specimens were received also from Picardy, Maryland, the twigs of the young trees having been injured severely by the Buffalo treehopper (*Ceresa bubalus* Fab.). Furthermore, the trees grown outdoors withstood infection to a much greater extent than did similar trees which had been transplanted into the greenhouse.

Ulster County, New York, and Suffolk County, Long Island, may be added to the places mentioned above where *Fusicoccum pyrorum* has been collected. Probably it is rather common but has been overlooked because of the other forms of the imperfect fungi found on the weakened or dying parts of the apple tree.

No attempts were made to apply control measures, but from the results of inoculation experiments, it would seem that the keeping of the trees in an actively growing condition, and free from injuries would be the two requirements necessary for keeping the fungus in check.

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DESCRIPTION OF PLATE XII

Fig. 1. Canker on branch showing discoloration of bark. The dead area is not depressed.

Fig. 2. Part of an infected twig, which had been placed in a moist chamber until the spores oozed in tendrils.

Fig. 3. Canker on young tree which had been inoculated with a pure culture. One infected twig was removed.

Fig. 4. Inoculated apple fruit, showing decay and stromata. The inoculations were made six weeks and nine months respectively, before photographing.

Fig. 5. Cross section of cankered branch showing depth of injury.

Fig. 6. Canker showing fruit bodies of *Cytospora* (the large, scattered projections on the left) and *Fusicoccum* (the smaller, more numerous ones on the right). The pycnidia of *Cytospora* are tipped with white, and come to a sharp point, while the pycnidia of *Fusicoccum* have rounded tops, and elongated slits.

Fig. 7. Cross section of pycnidium which is lined with conidiophores and pseudo-paraphyses.

Fig. 8. Photomicrograph of spores and pseudo-paraphyses.

Fig. 9. Cultures of *F. pyrorum* and *F. viticola* showing the more rapid growth of the former. Both cultures were prepared at the same time.

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FUSICOCCUM CANKER ON APPLES

THE RELATION OF SOIL MOISTURE TO THE DEVELOPMENT OF COMMON SCAB OF POTATO

G B SANFORD

The development of a scabby or clean crop of potatoes has almost always been explained on the basis of acidity or alkalinity of the soil, but there are numerous instances where scabby or clean crops of tubers have been harvested apparently regardless as to whether the soil received a treatment of acid or base forming chemicals, or no treatment at all. This indicates that soil reaction, after all, may not be the most important factor in influencing the development of the disease.

Another explanation often given is that the severity of scab may be directly proportional to the number of organisms in the soil, but the correlation does not seem to be consistent.

Extensive field observations and experimental work with common scab in Alberta suggested to the writer that soil moisture often might exert a strong influence on the development of scab. Experiments therefore were begun at the University of Alberta in 1921 and were continued in 1922. During both years the work was carried on under conditions as near those of the field as possible. For instance, plenty of room was allowed to plants, and ordinary untreated soil was used. In 1921 the plants were grown in boxes, 5' by 3' by 1'; and in 1922, large tin cans, 22" in diameter, each containing two plants, gave even better service. The boxes were lined with thick oil cloth to aid in controlling moisture, and water leads were arranged at the corners and bottom edges so that water might be evenly distributed from the bottom as well as from the surface of the soil. A dark organic sandy loam soil, common in Alberta, was used in the experiments. It had produced scabby crops the three preceding years and evidently was heavily infested with *Actinomyces scabies* (Thax.) Gussow. No inoculum was added. In 1922 a part of this soil was thoroughly mixed with surface soil from the University Experimental Plots which had grown one moderately scabby potato crop.

In order to get a greater temperature range (1921), one section was placed in the greenhouse where the plants grew to maturity under high temperatures during the months of July and August. The other section, a duplicate of that in the greenhouse, was arranged at the same time in the field where the soil boxes were neatly fitted into carefully dug trenches. This arrangement allowed the surface of each box to be

level with the ground so that approximately field temperature might be obtained in the soil. As it was impossible to control temperature under these conditions, the best that could be done was to take daily temperature readings at a depth of 4 inches.

Three series were run: dry, medium, and moist. On an air dry basis these represented a moisture content of about 12, 18 and 28 per cent respectively. The dry soil held too little moisture to produce a good crop, the medium was considered optimum, and the moist soil contained too much to permit normal growth of the plants. In 1922 5 series were tried (A, B, C, D, E). On an oven dry basis, the percentage moisture content of each series was: A—8, B—14, C—22, D—29, and E—34.

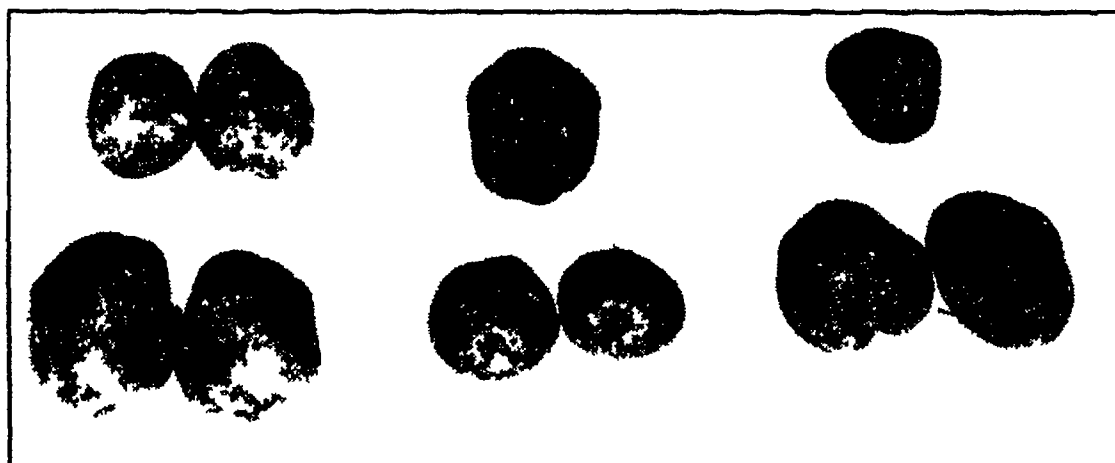


FIG. 1. Tubers from dry, medium, and moist soils in 1921. (Right to left.) Upper row grown in greenhouse; lower row grown in field, section of experiment.

The moisture content of A was so low that no tubers were produced; B allowed a fair growth of plant and tubers; C was better than normal field conditions; and both D and E might be considered too moist for good cultural conditions. The moisture content was kept nearly constant by daily weighings in 1922, but in 1921 water was applied according to frequent soil moisture determinations.

1921 RESULTS

The relative amount of scab produced in the dry, medium, and moist soils is shown in figure 1. Badly scabbed tubers were produced both in the dry soil and in the medium, but the potatoes in the moist soil were practically clean. Scab developed exceptionally well in the field where the soil temperatures ranged from 14.5° C. to 19° C. These figures indicate that soil temperatures in Alberta are not low enough to prevent the development of scab.

1922 RESULTS

The 1922 findings are similar to those of 1921. Figure 2 shows, in duplicate, the relative amount of scab produced in each series, excepting A, in which no tubers were formed because of insufficient moisture. In all cases the worst side of the potato is represented. Actual counts of scab spots are given in table 1.

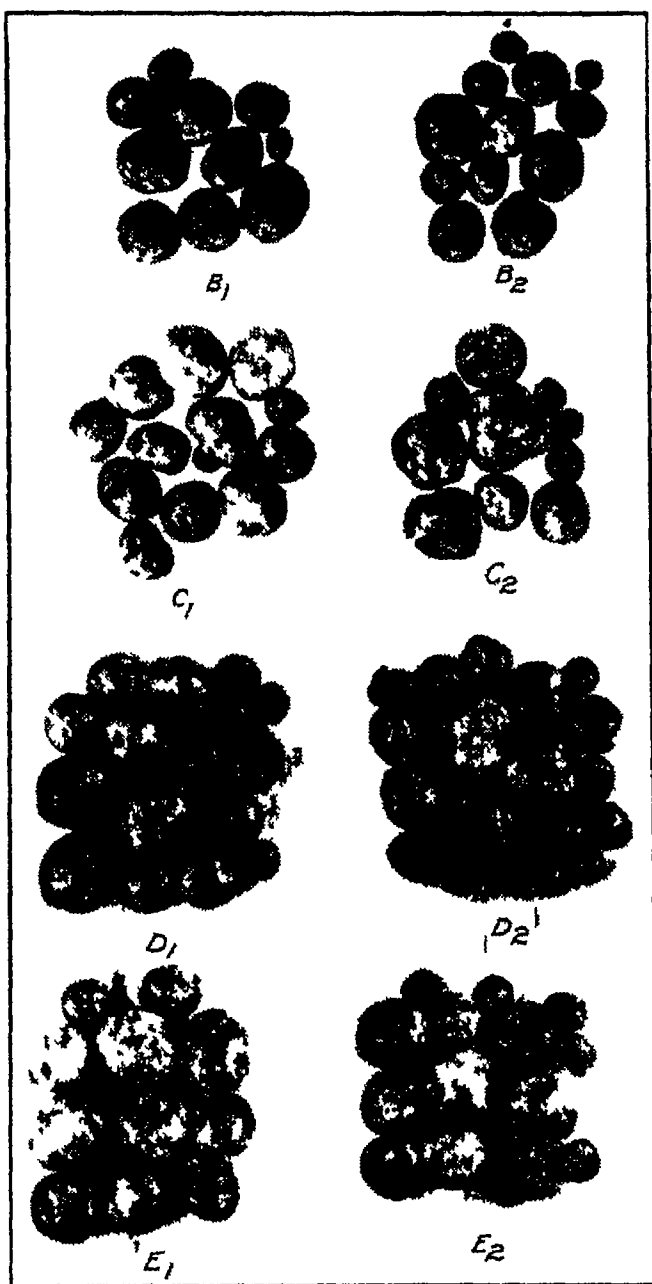


FIG. 2. Duplicate soil moistures of B, C, D and E, showing the effect on scab development in 1922.

TABLE 1—*The effect of different percentages of soil moisture on the development of scab.*

Percentage Moisture	Soil	Large (spots)	Small (spots)	Deep	Total
14	B ₁	26	448	10	474
	B ₂	83	558	27	641
22	C ₁	7	4		11
	C ₂	7	4	3	11
29	D ₁	1	0		1
	D ₂	6	13		19
34	E ₁	2	10		12
	E ₂	2	8		10

From the figures of table 1, it is evident that the soil contained an abundance of the scab organism and that moisture appears to have controlled, in some manner, the development of scab on the tuber.

Since the amount of scab has generally been attributed to the basic or acidic condition of the soil, hydrogen-ion determinations were made at intervals during the season by means of Leeds-Northrup type "K" potentiometer. The results are given in table 2.

TABLE 2—*The hydrogen-ion concentrations at different dates in soil containing different amounts of moisture.*

Percentage Moisture	Soil	July 28	Aug. 23	Sept. 7	Sept. 17
8	A	7.7	6.1	6.6	6.8
	A	7.7			
14	B	7.7	6.1	6.8	7.1
	B	7.7			
22	C	7.7	6.7	6.9	7.2
	C	7.7			
29	D	7.6	6.9	7.0	7.2
	D	7.7			
34	E	7.7	7.1	7.1	7.2
	E	7.6			

The experiment was started June 10 and tubers formed about July 25 and grew until September 17. It will be seen from table 2 that the pH values in the different soils were nearly the same on July 28; on August 23 the soils were slightly acid, while on September 7 and 17 there was a return to neutrality or slight alkalinity. These figures indicate that dry soils actually are more acid than moist soils under these conditions, and that hydrogen-ion concentration increased as the tubers grew. Apparently moisture, not acidity, was the controlling factor since the dry soil produced the scabby tubers and the more alkaline soil nearly scab-free potatoes.

An experiment was made to ascertain at which stage in the development of the tuber moisture exerts the greatest influence on the development of scab. While results were not entirely conclusive, it was clearly evident that if the soil is moist for a short time after the tubers are set they will not become scabby even after the soil becomes dry later in the season. This is shown clearly in table 3. The potatoes were planted in soil containing about 34 per cent moisture in large cans on June 10. Two cans were run in each series. The soil was all kept moist until June 26 when the soil in the first series was permitted to dry until a water content of 14 per cent was reached. Ten days later the next of the series was dried, and so on until August 25 when all had been dried. Results given in table 3.

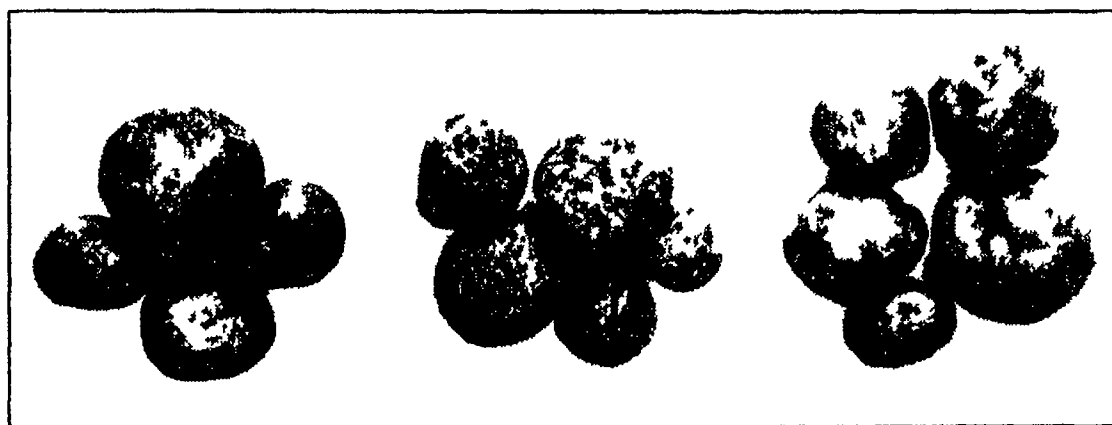


FIG. 3. Tubers from the dry soil which became accidentally wet during early tuber growth.

TABLE 3—*Scab development in relation to soil moisture at different periods of tuber growth.*

Soil	Date dried	Number of infections
1	June 26	111
2	July 6	312
3	July 16	220
4	July 26	3
5	July 31	46
6	August 5	9
7	August 15	0
8	August 25	4

Relative to moisture and the critical period for susceptibility, an interesting accident in 1921 seems worthy of mention. During the early development of the tubers, as noted at the time, a sudden down-

pour of rain choked the drain provided, and the water ran under the canvas shelter and into both ends of one of the dry soil boxes completely soaking the ground at the ends. Five days later this occurred again. Both times the moisture did not reach the middle hill to any extent. The soil became dry again and at harvest the end hills were surprisingly free from scab, while the middle hill was very scabby. Figure 3 shows the hills as they came from the soil.

SUMMARY

A study of the effect of soil factors on the development of common scab of potato has been made for two years at the Department of Field Husbandry, University of Alberta. The following conclusions seem justified.

Moisture appears to be directly or indirectly the main soil factor controlling the development of potato scab in the type of soil used.

Abundant scab developed in very dry soils, while soils sufficiently moist produced almost clean tubers.

The hydrogen-ion concentration of dry soils, which grew scabby potatoes, was slightly greater than that of the moist soil throughout the season. Scab developed abundantly in a hydrogen-ion concentration of about a pH 6 to 7.

There seems to be a critical period for susceptibility in the growth of the tuber, after which very little scab infection takes place.

A count of the *Actinomyces* in the soil used, and in others, shows that the organisms are very abundant in Alberta soils.

The study of this problem is being continued in the Division of Plant Pathology, University of Minnesota.

UNIVERSITY OF ALBERTA.

AN OUTBREAK OF HOPPER BURN IN VERMONT

B. F. LUTMAN

WITH ONE FIGURE IN THE TEXT

In spite of a very cool and rainy summer, leaf hoppers have appeared in considerable numbers on the potato plants at Burlington, Vermont, and have induced the very characteristic appearance on them described by Ball (1) in 1918 for the middle-west region of this country. The burns were first noted about August 15 on some Green Mountain plants brought into the laboratory for another purpose. The very cool season has prevented much tip burn of the physiological type so that an opportunity has been given this summer to see practically pure "hopper burn." The potato foliage does not seem to have attained the dark green color so characteristic of it, and these yellow-green leaves and plants have died early without the wilting and gradual advance during hot brilliant days which the author has described in other papers (3, 4). A study of the leaves cleared according to the alcohol-potassium hydroxid glycerin method (4) has shown that the water pores and the tissues surrounding the water chambers are not brown and dead as was evident according to the observations of last summer but are apparently functioning normally. A few exceptions were apparent under the water pores at the extreme points of some of the leaflets where this apical portion had apparently succumbed to drought, but in general the marginal tissues seemed to be healthy.

Some leaf hoppers were noted during July, but during early August they became so numerous that a few of the young hoppers were found on every plant. The hopper burn was not seen, however, until a little later, when a few rather warm days with a fair amount of sunshine brought it to notice. The hopper burn has been so carefully described by Ball that it is hardly necessary to restate the symptoms here, but in view of the fact that the author has worked with the other phase of the disease, it may be worth while to record the advance in death of parts of the leaves as they appear to a plant pathologist.

1. The most striking difference is first noted in the portions of the leaf involved. The veins and their adjacent tissues become brown to the unaided eye in a V-shaped wedge of diseased tissue. It will be discovered that the browning extends much further along the fine veinlets if the leaflet is cleared and examined under the low power of the compound

microscope, but it seems not to extend beyond the veins themselves. The sieve-tubes and companion cells of these veinlets are brown but along the larger veins, the long parenchyma cells which enclose them seem to be dead as is often the case with the tissue of the leaf itself. The striking point, however, to a student of physiological tip burn is that the vascular system leading from the mid-rib is the centre of the disturbance. The death is from the centre of the leaf outward while, in physiological tip burn, the reverse is true.

2. The second most striking difference lies in the portion of the leaflets involved. Tip burn would be a misnomer for hopper burn as any portion of the leaf might be involved and destroyed by the attacks of the insects and the tip is not more susceptible than the basal portions. In fact the insect may attack and destroy the main vein of the leaflet leading to the tip and the tip still remain green because the water continues to flow into it through the marginal plexuses described by the author in a previous paper. Figure 1, D illustrates this point. The mid-rib had collapsed and was brown as was also the surrounding tissue for an area of about a half a centimetre, but the extreme tip was unaffected. The same thing is shown in figure 1, A where the side of a leaflet died but the tip did not. Such a condition would not occur in ordinary tip burn of the physiological type.

It is very evident that in many instances it is almost impossible to distinguish the two forms of injury since they occur simultaneously. A careful examination of a number of the leaflets will make it fairly clear as to the factors involved if the two differences noted above are kept in mind. The entomologists have in some cases assumed that only one type can occur.

3. The seriousness of hopper burn as compared to physiological tip burn depends on the character of the weather during the summer in which the study is made. During the summer of 1921, when many plants in Vermont fields were almost denuded of their foliage by tip burn in a few weeks, the leaf hoppers must have played a very minor role, but during the summer of 1922, the bulk of the injury was of insect origin. There seems to be no question that the leafhopper can kill the leaflets even in cages and under greenhouse conditions where the sunlight is so weak that it will only rarely bring about a scald or burning in a few leaflets. In a hot, dry period, however, the sunlight may remove 30 to 50 per cent of the foliage in a week by causing extreme plasmolysis and pronounced wilting of the tips and margins. It would seem, therefore, to be a problem for the plant pathologists each year in the potato growing regions to note the advance of tip burn and to collaborate with the entomologists in determining the relative amount of the two types of injury.

Has the leaf hopper spread from the western states eastward in a similar manner to the Colorado potato beetle? How does the leaf hopper work on the leaflets?

The spread of the leaf hoppers if it occurred is probably very recent—a fact which should be ascertained before the evidence is lost. A small

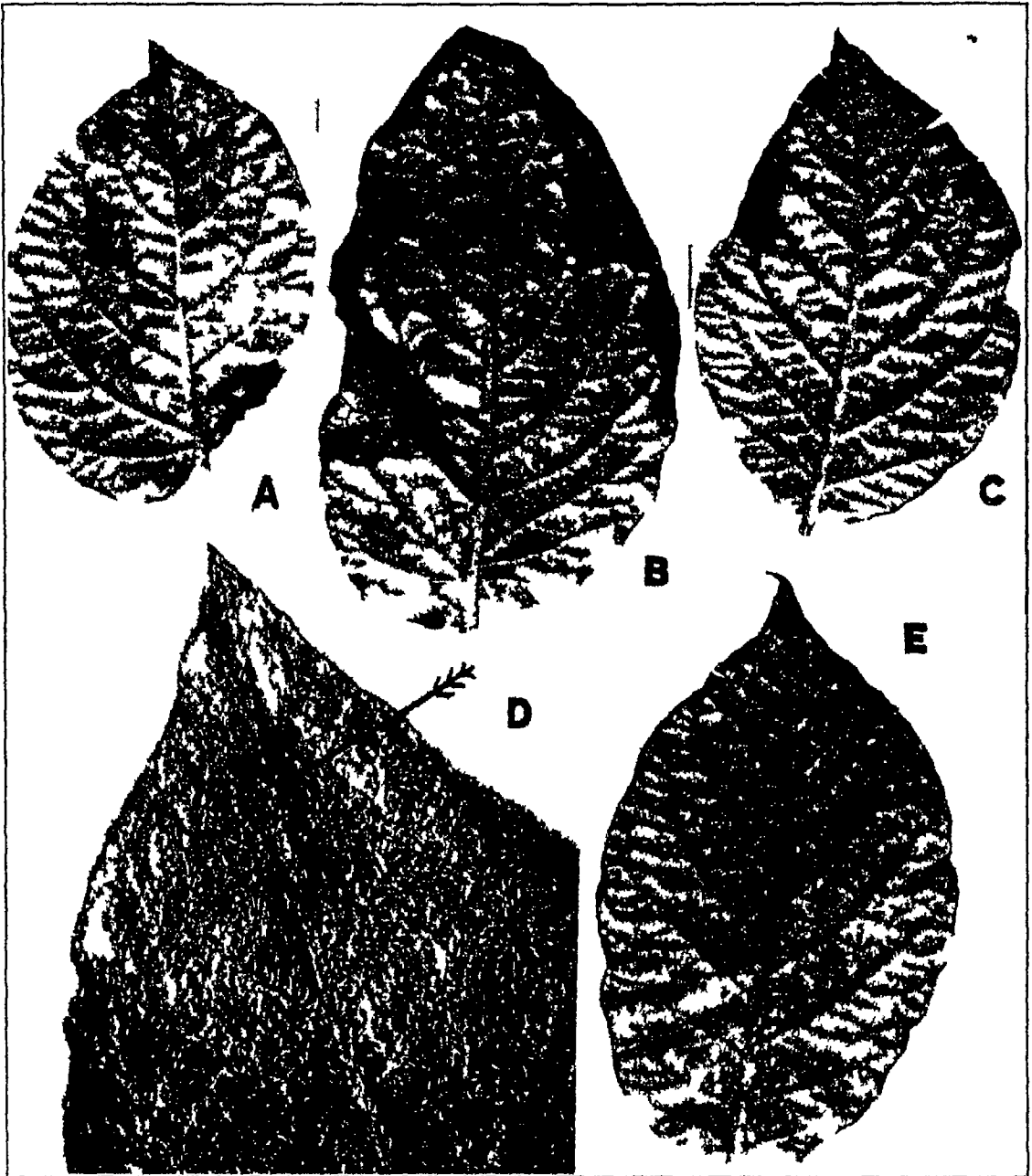


FIG 1 Characteristic hopper burn. Its early stages are shown in leaves A, B, and C. The mid rib of leaf D (much magnified) is collapsed the surrounding tissue being brown and dead. The tip is still green except at the extreme point. Leaf E seems to be in the early stages of physiological tip burn especially on the left side.

inconspicuous insect of this type could gradually make its way eastward without being noticed. The early descriptions of tip burn undoubtedly dealt only with the physiological form and the introduction of the hopper has injected quite a new factor into the problem.

The means whereby such small insects bring about such an almost incredible amount of injury in so short a time should be determined. The entomologists do not seem to collaborate with the plant pathologists in this study. The earlier views of Ball that the injury was partly mechanical in the destruction of the veins and partly physiological in the withdrawal of water from the cells is probably more nearly correct than that of Eyer (2) who would have us believe that some toxin is exuded as the hopper pumps the juice from the leaves. The experiments of this investigator would seem to indicate some such substance but it is doubtful if such an assumption is really necessary. A careful study of a number of leaves cleared in glycerine frequently failed to reveal the exact spot where the leaf was punctured by the proboscis of the hopper although the general region of destruction could be seen. Ball and others have noted the tendency of the hoppers to sit and suck on the veins on the underside of the leaf. A more careful study should be made of the feeding habits. It seems very probable, that the proboscis is inserted over and over again into the leaf, the operation being repeated as often as the supply of juice becomes scanty.

The cells accompanying the vessels, both the sieve tubes and their companion cells, as well as a surrounding sheath of parenchyma, are all much elongated and plasmolyse much more readily than even the long palisade cells. This fact can be readily confirmed by using solutions of various strengths of potassium nitrate on leaf sections. It seems not unreasonable to assume, therefore, that the leaf hopper removes the water from the tubes and from the adjacent sieve tubes, companion cells, and elongated parenchyma to such a degree that they plasmolyse and collapse to a point where they cannot regain their turgidity, and as a consequence they die and become brown in color. The extent of injury about such an attacked region would depend upon the amount of water in the soil, on the warmth and brilliance of the sunshine and on the humidity of the atmosphere. This explanation would seem to cover the faces observed by a study of the leaves themselves and to obviate the assumption of toxic injection.

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POTATO SCAB IN NEW LAND

B. F. LUTMAN

Some years ago, Mr. G. C. Cunningham, then associate Plant Pathologist of the Vermont Agricultural Experiment Station, suggested to the writer that a study of the occurrence of scabby potatoes on new land that had not been previously cropped might throw some light on the suspected general distribution even in new soil of the scab producing organism. Mr. Cunningham left the Vermont Station before any experimental work was done in this connection but the author has carried out field trials during the past six years with a view of throwing light on this subject.

There is very little literature on the subject of the occurrence of any disease in absolutely new soil, apparently free in every way from the organisms associated with it. Plant pathologists seem so obsessed by the narrowest possible interpretation of the germ theory of disease that even the suggestion that many of the parasitic organisms are only common saprophytic ones whose mode of life has been altered, would be regarded as rank heresy. Every specific disease has its own specific parasite associated with it. Jones and Edson¹ at the Vermont Station in 1901 unintentionally have shown that potatoes may be scabby even though raised on new land. These investigators attempted to find land that contained no scab producing organisms in order to test the efficacy of seed treatment. Absolutely new land that had been recently cleared of pine and had not been previously cropped was planted with seed that had been soaked for one and a half to two hours in corrosive sublimate solution, one part in a thousand of water, or in formaldehyde, one part in two hundred and forty of water. It is generally assumed that this treatment is sufficient to kill all fungi of the *Actinomyces* type and laboratory tests confirm this assumption. In spite, however, of such treatment, the rows containing seed pieces soaked in the corrosive sublimate solution produced 2.75 to 5.9 per cent and those soaked in

¹ Jones, L. R. and A. W. Edson. Potato scab experiments of 1901. Vermont Agric. Exp. Sta. 14th Ann. Rpt., 1900-01: 231-235. 1901.

formaldehyde solution 1.50 to 10.66 per cent of scabby tubers. The only possible assumption of error for those who believe that the parasites are always introduced into virgin soil by scabby seed, is in the unreliability of the disinfection methods.

In 1916, four small plots of formaldehyde disinfected potatoes were planted in small spots, open to the sky, in the "College Woods," a white pine growth at least fifty years old and uncleared in the present generation.

TABLE 1—*Showing the percentage of scab and Rhizoctonia infected potatoes in four small plots, in soil which had not been cultivated for at least 50 years.*

	Disease	Tubers free from	Slightly scabbed tubers	Badly scabbed tubers	Per cent clean	Per cent slightly scabbed	Per cent badly scabbed
Plot 1 13 tubers ¹	Common scab	10	3	0	77	23	0
	Rhizoctonia	13	0	0	100	0	0
Plot 2 15 tubers ²	Common scab	9	6	0	60	40	0
	Rhizoctonia	0	6	9	0	40	60
Plot 3 110 tubers ³	Common scab	56	36	18	51	33	16
	Rhizoctonia	76	22	12	69	20	11
Plot 4 38 tubers ⁴	Common scab	11	16	1	39	57	
	Rhizoctonia	0	16	12	0	57	43

¹ Four hills in sandy, dry soil on a hill side.

² Four hills in very dry, sandy soil on top of a hill.

³ Fifteen hills in sandy loam.

⁴ Six hills in sandy loam with much forest humus; in a shady situation; yield small.

The work was discontinued during 1917 and 1918, owing to lack of assistance, but experimental work was again attempted in 1919, when about an eighth of an acre of clean, disinfected Green Mountain potatoes were planted in a piece of land cleared of pine during the preceding winter. No commercial fertilizer was put in the hills. Many of the pine roots and stumps were still in the plot. The soil was very sandy but with an abundance of forest humus. The plot was in a very low lying situation and in the early spring the water table was not over a foot from the surface of the ground. An examination of several bushels of the tubers produced showed between one half and one per cent of

them with typical deep scab spots. In the majority of cases these lesions were single but occasionally an entire end or side of a tuber was covered by the brown corky growth.

In 1920, part of the same land was planted with clean disinfected Irish Cobblers, and some rows were placed in a new strip that had been cleared of pine during the winter. A commercial potato fertilizer was added to the hills. The count showed 93 per cent of clean tubers and 7 per cent of scabby tubers on the land used the year before and 97 per cent of clean and 3 per cent of scabby on the potatoes from the virgin soil. The new land was a little lower and wetter than the old portion.

In 1921, the same land was again employed for these experiments. A strain of Green Mountain seed, clean, but also disinfected with formaldehyde was planted and commercial fertilizer was added to each hill. When the plants were dead,—Sept. nineteen,—and the rows dug, it soon became apparent that the amount of scab in various parts of the long narrow plot varied greatly so the results were compiled in three parts.

	Clean	Slightly scabbed tubers	Badly scabbed tubers
	per cent	per cent	per cent
South end	40	43.	17.
Middle	87.	12.	1.0
North end	83.	15.	2.
Average for entire plot	71.	22.	7

The two ends of the plot were apparently the same soil and had received identical treatment. Poured agar plates showed an abundance of organisms of the *Actinomyces chromogenus* type present in the north end.

The experiments made in 1919, 1920, and 1921 illustrate the rapid increase in scabby potatoes on land continuously cropped with potatoes. The occasional scabbed tuber of 1919 increased to 60 per cent of the crop at the south end of the plot in 1921, even when all precautions were taken against the introduction of parasitic strains of the seed tubers or in the manure. It would hardly seem possible that the few lesions found in 1920 would so infect the soil that almost every tuber in growing would come in contact with a bit of parasitic mycelium unless the roots of the potato were covered with it. It would seem more reasonable to suppose that the particular methods used in potato tilth give the organisms of the scab type a chance to come to the front in the destruction of the cellulose in the soil humus and that parasitism is entirely secondary.

The same plot was again planted in 1922, but the very rainy June (9.92 inches) caused every seed piece to rot. Four plots were also planted in another region in small openings in near by pine woods. This land had never been under cultivation and the plots were surrounded by pine trees 30 to 50 years of age. The soil was light and sandy except in plot II (which was on low ground and a rather heavy clay). A commercial potato fertilizer was used. The plants were at a disadvantage from lack of light and the yield and the tubers were small. The following tabulation of the yields will show the rather high percentage of infection:—

	Number of hills	Tubers showing Rhizoc- tonia	Tubers clean	Tubers slightly scabby	Tubers badly scabby	Per cent clean	Per cent slightly scabby	Per cent badly scabby
I	16	4	63	24	6	68	26	6
II	4	3	17	2	4	74	17	9
III	9	0	7	3	1	70	30	10
IV	20	5	38	11	0	78	22	0

The scabs obtained on these tubers were typical and in some cases covered almost half the entire surface. The presence of *Rhizoctonia sclerotia* is also of interest and shows that new land is not necessarily clean land.

SUMMARY

Potatoes grown on land never cultivated will often develop typical common scab lesions and *Rhizoctonia sclerotia*, even if the seed tubers are disinfected and only commercial fertilizers are used.

DOTHICHIZA CANKER ON NORWAY POPLAR

FREDA DETMERS

WITH PLATE XIII

Poplar canker caused by *Dothichiza populea* Sacc. and Briard, has been reported in the United States (2) and Canada (3) on several species of poplar, notably *Populus nigra* and *Populus deltoides*. The latter may also cover the variety *P. monilifera* which according to Bailey (3) is the northern cottonwood. The author knows of no publication reporting this disease on the Norway poplar, *Populus eugenei* Simon-Louis. She wishes, therefore, to record the occurrence of *Dothichiza* canker on *Populus eugenei* in Ohio.

HISTORICAL SKETCH

In the spring of 1917, the Mead Pulp and Paper Company of Chillicothe, Ohio, set out 1200 Norway poplars in the bottom lands of Paint Creek, a tributary to the Scioto River. On May 5 of that year, Mr. Bearce, forester to the company, reported a bark disease of these poplars to the Department of Botany of the Ohio Agricultural Experiment Station. No definite conclusion was reached as to the identity of the disease at that time, but fear was expressed that it might be due to *Dothichiza populea*. During 1921 the attention of the department was again called to the case and a personal visit was made. Careful examination of abundant material has established the identity of the causal organism to be *Dothichiza populea*. This diagnosis has been corroborated by cultures, and was verified by Dr. George G. Hedgcock to whom I sent fresh material. In 1921, the company had five blocks of Norway poplars ranging in age from one to six years all severely infected. The disease has been so destructive that the company has decided to abandon the Norway poplar and during the past winter blocks of one and three and four year old trees have been dug out and burned. In the six year old block of trees the disease has progressed less rapidly during the past season. It is possible that these trees may recover sufficiently to be worth saving.

FIELD OBSERVATIONS

Infection takes place at the nodes. The discolored area may be immediately below the leaf scar, in the axil of the bud, or on either side of the bud in proximity to the stipular scars. The bud itself is not attacked. Cankers form at any node on two year old wood. The lowest

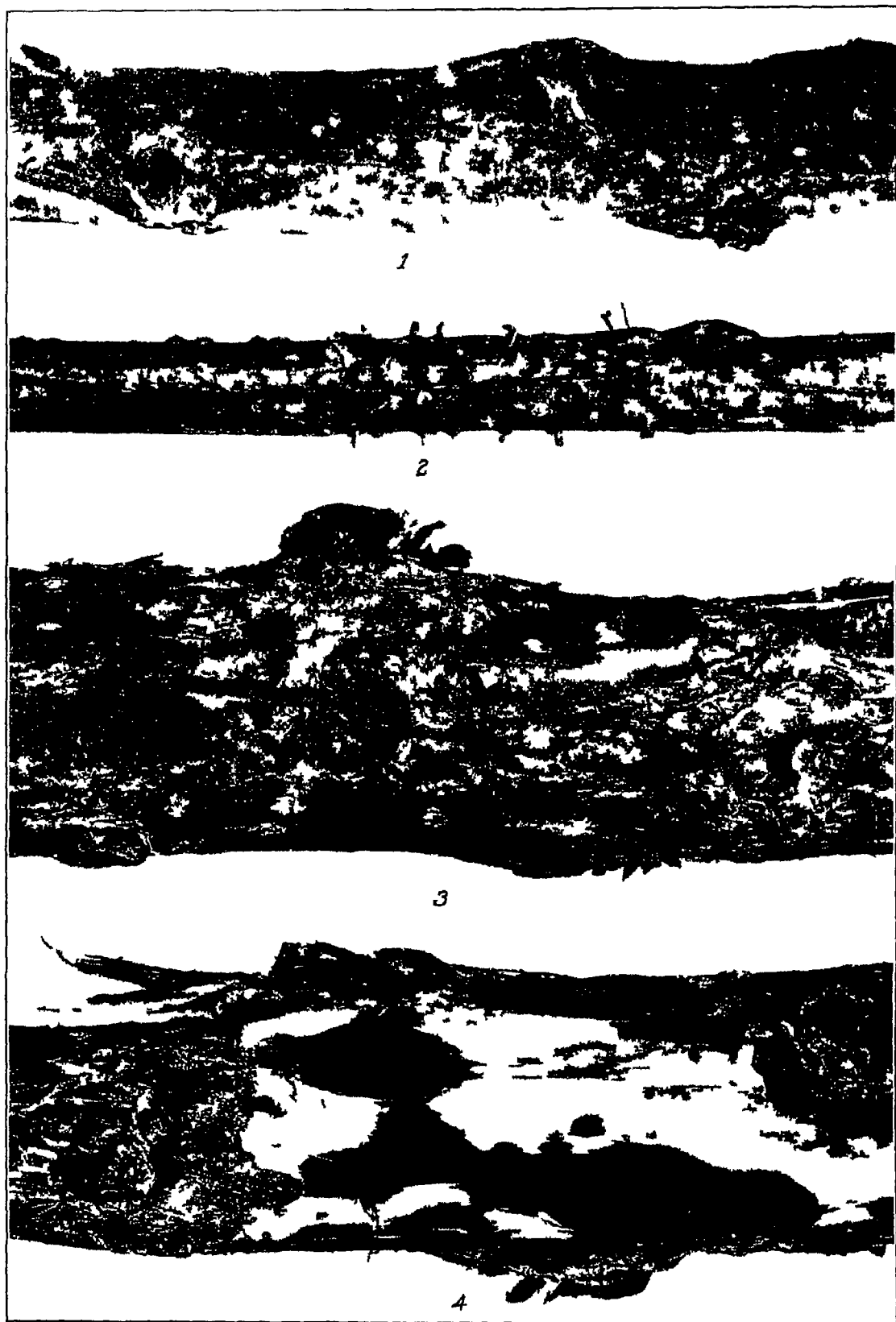
found was on the trunk of a four year old tree, three inches from the ground. The annual spread of infection has generally been slower than the growth of the trees and has not extended beyond one year old wood; however, in severely diseased young trees the current-year's growth was also invaded. A large branch of one of these trees was surrounded at its base by a pronounced canker. The branch had thirty-two nodes, (Pl. XIII, 2) of which the fifth, sixth, ninth, eleventh, sixteenth and eighteenth nodes were infected. The relative age and severity of attack indicated that the trend of infection had been upward from the lower to the higher nodes. Numerous pycnidia were scattered (Pl. XIII, 1) over infected areas on the young branches, and from them the characteristic broad rather short spore threads emerged. Cankers on older branches (Pl. XIII, 3) are rough, the bark becomes cracked exposing in severe cases the wood beneath. The sap wood is invaded (Pl. XIII, 4) becoming deep shining brown as shown in the illustration. Lateral branches from infected areas are killed. The dead lateral branches are a striking feature of the presence of the disease. Cankers spread laterally, finally coalesce and girdle the main trunk. As the spread of invasion is from below upward the upper buds remain healthy and often make a vigorous growth of ten feet in one season. This rapid growth is characteristic of the Norway poplar.

THE NORWAY POPLAR

A brief statement of the identity of the tree known to nurserymen as the Norway Poplar or "Sudden Sawlog" may not be amiss as it seems to be but little known. I am indebted to Dr. George B. Sudworth (4), Dendrologist, United States Forest Service, for the information:—that the tree is a hybrid between a European-grown form of our common eastern cottonwood *Populus deltoides* and the European black poplar, *P. nigra*. This hybrid was described and named in Europe many years ago as *Populus eugenei*. Dr. Sudworth adds:—"Another name for it is possibly *Populus robusta*, which first appeared in French literature, this form having originated in a French nursery and is believed to be from the same parentage as *Populus eugenei*. Doubtless the French form was grown in Norway where the name Norway Poplar was applied."

Bailey (1) says of *Populus eugenei* Simon-Louis, "This interesting and valuable, rapidly growing poplar was found in 1832 as a chance seedling in the Simon-Louis nursery, near Metz, France; it is supposed to be a hybrid between Lombardy poplar (male) and *P. regenerata* (female)."

C.S. Harrison, a nurseryman of Falling Water, Nebraska, is accredited with having introduced the tree into America.



DOTHICHIZA CANKER ON NORWAY POPLAR

The tree is similar to *Populus nigra* in its strict habit of growth, narrow crown, dark bark and failure to clean up the trunk through self pruning. The shape of leaf blade and bud are similar to that of *P. deltoides*. It grows much more rapidly than either parent and because of its rapid growth it is deemed of value as a source of wood pulp, for it can be cut several years earlier than other poplars. The poplars stand first among the hard woods suitable for wood pulp; and because of its rapid growth the Norway is preferred to other species of poplars, consequently the extreme susceptibility of this tree to *Dothichiza* canker and the virulence of the disease is a blow to the wood pulp industry in Ohio.

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A MELIOLA DISEASE OF CACAO

F. L. STEVENS AND RUTH I. DOWELL

WITH THREE FIGURES IN THE TEXT

During an inspection of cacao plantations at Coverden, British Guiana, for pod rot and witches broom a leaf-spot disease evidently due to *Meliola* was seen in considerable abundance and collected. The disease as manifest in this plantation cannot be considered as serious though it is conceivable that under especially favorable conditions it might become so.

Of the numerous species of *Meliola* those that cause appreciable killing of tissue are in small minority. *Meliola* was long considered to be merely a superficial fungus; then it was demonstrated that it sends numerous haustoria into the epidermal cells, and usually without apparent effect on the host. Now, however, several species of the genus are known to produce dead spots coincident, or nearly so, in size with the superficial colony, the spots so produced showing as dead areas from

both sides of the leaf. Such is the case in the present instance and spots of dead leaf tissue 3 to 10 mm. in diameter are produced. The *Meliola* proves to be a new species and is described as follows:

***Meliola guianensis* n. sp.** (Figs. 1, 2 and 3A to 3D.)

Fungus hypophyllous. Colonies 3–10 mm. in diameter, nearly circular; the spots visible from the opposite side of the leaf.

Perithecia about 280 μ in diameter, somewhat warty. Setae dark, spiral, echinulate on the upper one-third to one-half of length, about 86 μ long $\times$ 7 μ thick. Asci evanescent. Spores 4-septate, 54 $\times$ 19 μ , dark. Mycelium slightly sinuous, branches opposite, sometimes alternate. Capitulate hyphopodia alternate, rarely opposite. Mucronate hyphopodia opposite and alternate. Mycelial setae absent.

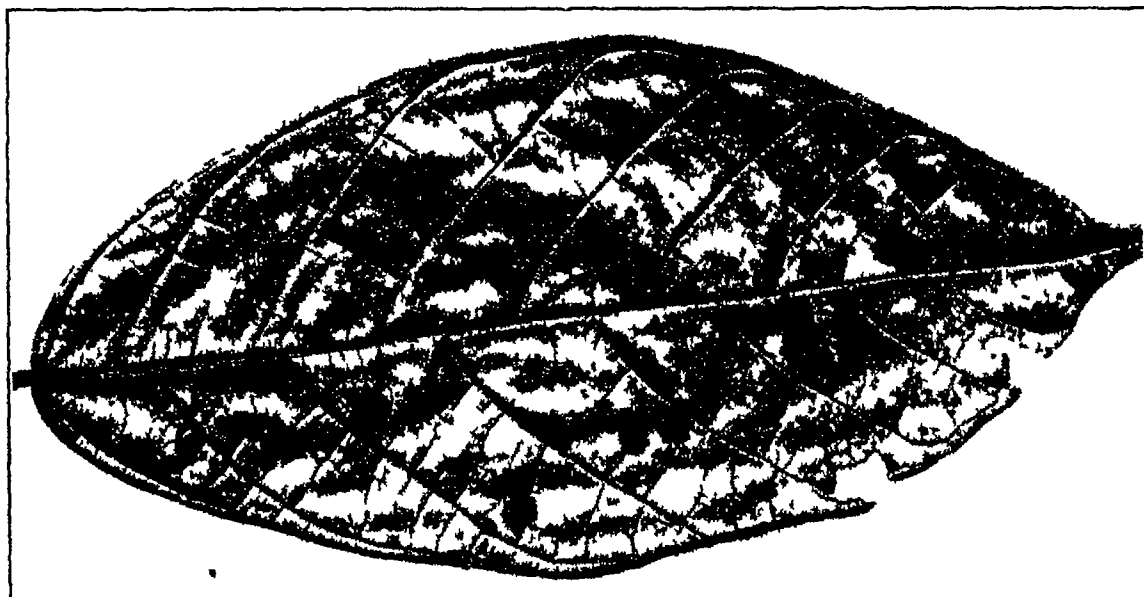


FIG. 1. Leaf of *Theobroma cacao* showing distribution of colonies of *M. guianensis* $\times$ 1; reduced 1/3.

Group number 3301.5231.

On *Theobroma cacao*. British Guiana; Coverden, August 4, 1922, number 974.

The group number given above is based on the system of Beeli¹. The species falls within a small group of the genus characterized by the possession of 4-septate spores, perithecia with setae, no mycelial setae and alternate hyphopodia. There were only seven such species previously known and with only one of these, viz. *M. tortuosa* Wint., is it in

¹ Beeli, M. Note sur le Genre *Meliola* Fr. Bull. Jard. Bot. Bruxelles 7: 91. 1920.

at all close agreement. From this species, however, it differs markedly in that the perithecial setae in the present species are distinctly echinulate in their distal portion, (Fig. 3A and E); also the perithecia (about $280\ \mu$ in diameter) and spores ($54 \times 19\ \mu$) are larger than those of *M. tortuosa* which are respectively $160\text{--}190\ \mu$ in diameter and 31 to $36 \times 12\ \mu$ (Fig. 3B and F); the setae and mycelium are opaque and darker than those of *M. tortuosa*; and the mycelium is somewhat larger and less sinuous (Fig. 3C, D and G).

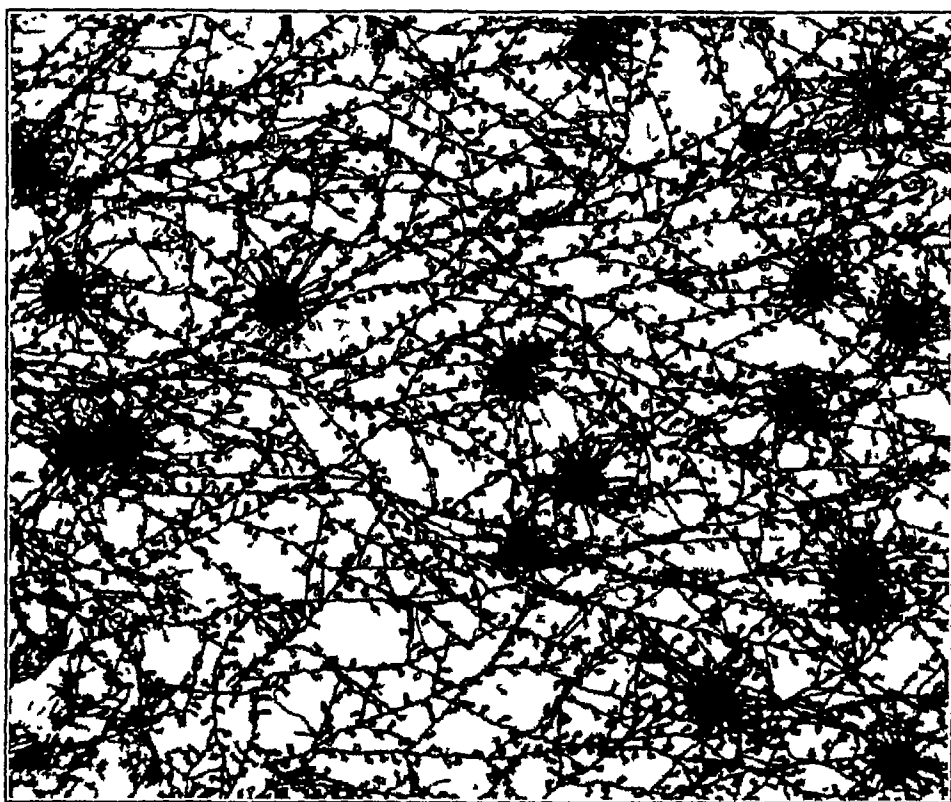


FIG. 2. Photomicrograph of *Meliola guianensis*, portion of colony showing mycelium hyphopodia and perithecia.

Meliolinopsis theabromae Beeli (syn. *Meliola theabromae* F.C. v. Fab.) having a spore length of only $10\ \mu$ and with 8-spored asci is very different.

The *Meliola* was overgrown in some instances by a *Helminthosporium*, in others by a *Nectria*, the former rendering the colony unusually black and the latter making it white, thickly strewn with the pink perithecia. The *Helminthosporium* is new and is described as follows:

***Helminthosporium guianensis* n.sp. (Fig. 3H.)**

Mycelium fine ($1.5\ \mu$) pale straw-colored, conidiophores thicker ($3\ \mu$), pale brown, occasionally branching, $68\ \mu$ long. Conidia 3-septate, $18\text{--}21 \times 3\text{--}7\ \mu$, thicker in the middle and tapering toward both ends.

On *Meliola guianensis* Stev. & Dow. on *Theabroma cacao*. British Guiana, Coverden, August 4, 1922, number 974.

The *Nectria* appears to be *N. portoricensis* Stev., though it differs in that it does not coat the *Meliola* mycelium with a white mycelium. The perithecia are scattered throughout the colony and are pale flesh color rather than red; and the spores are hyaline.

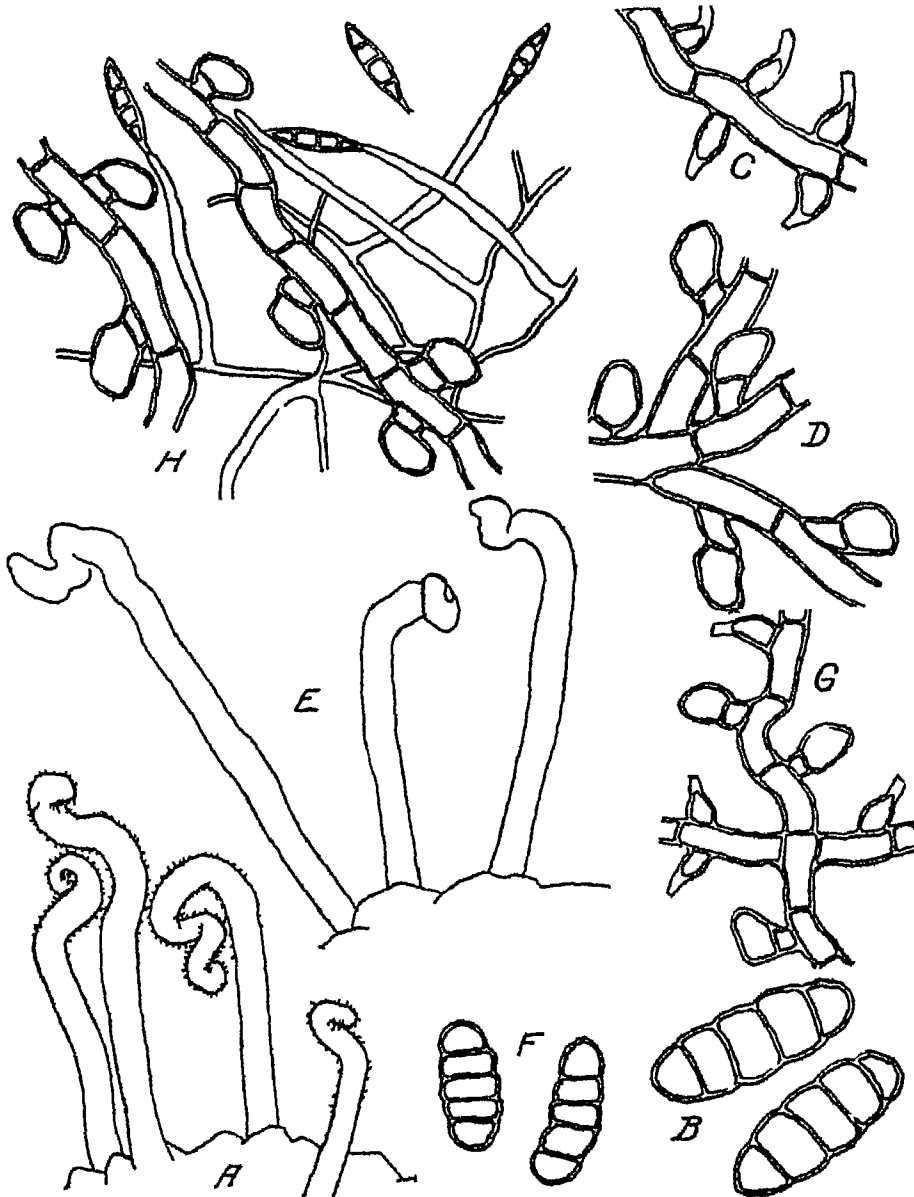


FIG. 3. (Drawn to a high power.) A, *M. guianensis*, surface of perithecia and echinulation of setae; B, *M. guianensis*, ascospores; C, *M. guianensis*, mucronate hyphopodia; D, capitulate hyphopodia; E, *M. tortuosa*, surface of perithecia and setae; F, *M. tortuosa*, ascospores; G, *M. tortuosa*, mycelium showing mucronate and capitulate hyphopodia; H, *Helminthosporium guianensis*, showing mycelium, conidiophores and conidia, and mycelium of *M. guianensis*.

THE HOT WATER TREATMENT OF CABBAGE SEED

J. C. WALKER

In a former paper¹ the writer has shown that the cabbage black-leg organism, *Phoma lingam* (Tode) Desm., can not be completely eradicated from internally infected seed, by any method tried, without more or less reduction in amount of seed germination. Inasmuch as different lots of seed vary widely as to the amount of injury sustained from stronger treatments, a moderate but safe formula was recommended for general use (mercuric chloride solution 1 : 1000 for 30 minutes). While the above limitations of seed treatment point to the ultimate control of black-leg through the production of disease-free seed, the fact still remains that cases arise from time to time in which complete disinfection is essential even at the expense of a moderate reduction in germination. This is especially true in the case of infected but otherwise very valuable stock seeds from which it is advisable to eradicate the fungus before they are planted for purposes of multiplication. It is a problem of primary interest to the commercial seed grower as well as to the experimenter in selection and breeding. In our own experience in selecting yellows-resistant strains of cabbage we have found it necessary to devise a method for complete disinfection of small lots of pedigreed seeds which had become infected by *Phoma lingam*. Several commercial seedsmen have also recently sought advice on the same question.

The procedure already outlined in the paper mentioned, was therefore continued in order to determine the most satisfactory method for complete disinfection. The dry heat and formaldehyde treatments being obviously out of the question the effects of stronger treatments with mercuric chloride and with hot water were studied further. It was soon decided, as may be judged from the data in Table I, that mercuric chloride was unsatisfactory for the purpose. Hot water gave more promise. It will be seen that complete disinfection was nearly reached with 15 minutes' exposure at 50° C. and was completely attained with 30 minutes' exposure at the same temperature. Moreover, the increase in temperature to 55° C. did not shorten the period necessary for complete disinfection.

Supplementary field tests with treated lots of infected seed under conditions very favorable for black-leg development, showed the mercuric-chloride treatment to be unsatisfactory and the hot water at 50° C. for 15 minutes insufficient. The longer hot water treatment (50° C., 30 minutes) yielded practically perfect control.

¹ Walker, J. C. Seed treatment and rainfall in relation to the control of cabbage black-leg. U. S. Dept. Agric. Bull. 1029. 27 p. 1922.

TABLE 1—*Effect of hot water and mercuric chloride treatments upon the viability of Phoma lingam in infected cabbage seeds.*

Number of seed lot	1		2		3	
Treatment	Number of seeds used	Seeds with viable fungus after treatment (per cent)	Number of seeds used	Seeds with viable fungus after treatment (per cent)	Number of seeds used	Seeds with viable fungus after treatment (per cent)
Control	117	60	29	52	24	67
Mercuric chloride { 30 min.	31	65	—	—	10	50
{ 60 "	30	47	—	—	22	64
1 : 500 { 120 "	30	30	—	—	25	4
Hot water { 50° C. 15 min.	163	0 6	19	0	—	—
{ 50° C. 30 "	141	0	20	0	15	0
{ 55° C. 15 "	—	—	—	—	35	3
{ 55° C. 30 "	—	—	—	—	24	0

The effect of these two lengths of exposure at 50° C. was studied with a number of seed samples of known age and origin as well as with several taken at random from a retail seedsman's list. From the data secured (Table 2) it will be seen that as a rule little reduction in germination was experienced with the shorter treatment. With the longer exposure (30 minutes) the reduction was more marked, and as a rule it was more

TABLE 2—*Effect of hot water treatment upon the germination of cabbage seed*

Variety	Approximate age of seed (months)	Percentage germination		
		Control	50° C. 15 min.	50° C. 30 min.
Wisconsin All Seasons	3	92	90	90
Wisconsin Hollander	6	93	86	85
Wisconsin Hollander	6	82	68	67
All Seasons	6	97	91	82
Wisconsin All Seasons	18	69	69	63
Wisconsin Brunswick	18	67	64	50
Wisconsin Hollander	18	65	47	38
Wisconsin All Seasons	30	70	63	47
Sure Head	Age unknown; samples taken at random from retail seedsman's list	89	—	61
All Head Early		81	—	67
Danish Ball Head		85	—	76
Dry Weather		81	—	49
Earliest of All		74	—	31
Early Summer		34	—	13
Late Flat Dutch		38	—	4

pronounced in the older seed. Retarding of germination was usually noticeable in treated lots and it increased roughly in proportion with reduction of percentage of germinating seeds. It is evident from these data that seed of high germination when treated at 50° C. for 30 minutes within the first six months after harvest may be expected to suffer only moderately in reduction of germination. The injury may be expected to increase with the age of the seed at time of treatment. The variability in the results secured with seed lots picked at random is therefore to be expected.

For the special cases already outlined, where complete freedom from the black-leg fungus is urgently needed, the treatment with hot water at 50° C. for 30 minutes is practicable. We have used it satisfactorily for the past two years on seed lots varying in size from a few grams to several pounds. The limitations of the treatment should be recognized, however, and preferably preliminary trials with a few seeds should be made. The variability in injury sustained by seed lots picked at random preclude the recommendation of this treatment for general use.

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BANANA WILT OF THE MANILA HEMP PLANT

H. ATHERTON LEE AND F. B. SERRANO

Manila hemp is regarded among fiber men as unequalled among plant fibers for the manufacture of high grade cordage such as that used on ships and for other marine uses. It is obtained by a stripping process from a plant closely resembling the banana plant in appearance, a member of the same genus, *Musa textilis* Neé. The Manila hemp plant is confined in its distribution to the Philippine Islands and has never to the present time been successfully cultivated commercially in any other country. The Philippine Islands therefore hold a monopoly on the production of the very superior plant fiber, Manila hemp.

A disease of the Manila hemp plant was described in 1915, by Reinking¹ as abaca² heart rot and its cause ascribed to bacteria, the identity of

¹ Reinking, O. A. Philippine economic-plant diseases. Philippine Jour. Sci. Sec. A. 13: 221. 1918.

² Abaca is the dialect word in the Philippines for the Manila hemp plant.

which were undetermined. More recently, this disease has increased in seriousness and investigations were requested by commercial growers. The distribution of the disease was first determined and it was found to be destructive only in the provinces of Laguna and Cavite.

Isolation and inoculation studies were undertaken with the expectation of corroborating earlier results as to the cause; bacteria were found in isolation studies to be commonly associated with advanced stages of the disease, although an imperfect fungus was almost as uniformly isolated. As familiarity with the disease became greater it was possible to find less advanced pathological stages and from such earlier stages, a *Fusarium* was obtained in a very high percentage of cases. Examination of fresh frozen sections and of serial paraffin sections showed with little or no difficulty the presence of fungus hyphae in the early advancing stages of the disease. Isolations were continued repeatedly, in all 1468 isolations being made; after the first four series of isolations, *Fusarium* began to appear and from that time thereafter appeared in 60.44 per cent of the plantings. Few fungi of other genera appeared in such plantings. The bacteria isolated in the older stages did not appear uniformly although three different forms of colonies on beef agar plates appeared more frequently than others.

The isolation attempts having reached this stage, inoculation experiments were tried. The bacterial species for convenience were termed organisms A, B, and C. Since the bacterial species ascribed as the cause of the disease by Reinking was not described, its identity with any one of these three organisms could not be determined. Inoculation studies have been made with all three of the bacterial species and with the *Fusarium*. Inoculations were made with needle punctures, through the pseudostem into the central cylinder. Table 1 shows the number of such inoculations and results.

It seemed apparent to us, therefore, that the *Fusarium* species was a pathogene.

Studies were then taken up to identify this *Fusarium* species. We had recently isolated *Fusarium cubense* Erw. F. Smith, from banana wilt cases in the Philippines and the hemp plant fungus was being cultured at the same time. Comparisons were naturally made of morphological and cultural characters and it was found impossible to distinguish the two fungi in this way.

In earlier studies the banana wilt fungus inoculated into banana plants caused blackened discolored rhizomes, splitting of the pseudostem and yellowing and dying of the leaves. The same organism inoculated with

TABLE 1—Showing results of inoculations into the central cylinders of the Manila hemp plant, with various organisms isolated from heart rot cases. Sterile needle punctures used as controls.

	Number of inocu- lations	Number of positive infections	Percentage of positive infections
Organism A.	20	1	5.00
Organism B.	80	8	10.00
Organism C.	145	9	6.20
Sterile needle puncture through sterile ex- ternal surface.	70	4	5.71
Hemp Plant Fusarium	199	96	48.24 ¹

the same technique into the pseudostems of Manila hemp plants did not cause such lesions and the hemp plant was at the time considered resistant.²

Inoculations were made with needle punctures, through the thick pseudostem, into the central cylinder of Manila hemp plants with the banana wilt Fusarium, and with the Manila hemp plant Fusarium. The banana wilt Fusarium gave 65 infections in 115 inoculations, or 56.5 per cent; the Manila hemp Fusarium gave 89 out of 134 attempts or 66.4 per cent.

Since the morphological and physiological characters of the hemp plant pathogene seem to be similar to those of the banana wilt organism, *Fusarium cubense*, the two must be considered for the present at least, as belonging to the same species.

The conclusions moreover are, that *Fusarium cubense* can not produce banana wilt symptoms upon the Manila hemp plant, but if inoculated into the growing central cylinder of the hemp plant by means of the proper technique, it can produce typical heart rot. The interesting situation is developed of a fungus species producing one set of symptoms on one host plant, and an entirely different set of symptoms on another.

Only two banana plants have been inoculated with the Manila hemp Fusarium, but both plants have given banana wilt symptoms, probably

¹ As the technique was improved, positive results were obtained with the hemp plant Fusarium in 89 out of 134 attempts or 66.41 per cent. Failure to obtain 100 per cent positive results possibly may be correlated with the improbability of placing the inoculum, through the thick resistant pseudostem into the susceptible central cylinder tissues in every inoculation attempt.

² Lee, H. A. and F. B. Serrano. Banana wilt in the Philippines. *Phytopath.* 10: 504-505. 1920.

more promptly and virulently than would *Fusarium cubense* direct from banana wilt. A control puncture remained healthy.

Our knowledge of heart rot of the Manila hemp plant is, therefore, greatly increased since, from the work of Brandes¹ it is known that *Fusarium cubense* is not a strict parasite. It can, however, exist in the soil and infection can take place from that source. The discovery of this fact leads also to an understanding of the heart rot of the hemp plant which may become just as destructive as is the banana wilt in the West Indies.

The most promising means for the control of the disease seems to be with resistant varieties. A more detailed report will appear in the Philippine Journal of Science.

PHILIPPINE BUREAU OF SCIENCE,

PHILIPPINE BUREAU OF AGRICULTURE

¹ Brandes, E. W. Banana wilt. *Phytopath.* 9: 339-389. 5 fig., pl. XXI-XXXIV (partly col.) 1919. Literature cited, p. 388-389.

Errata: February number of *Phytopathology*, page 99, line 5, instead of "one plant" read "all the descendants of one."

Page 38, article by V. F. Tapke, last line instead of "controls loose smut of wheat reducing yields" read "controls loose smut of wheat without reducing yields".

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THE PHYSIOLOGY OF PERITHECIAL AND PYCNIDIAL FORMATION IN VALSA LEUCOSTOMA

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Although considerable work has been done on the physiology of reproduction in fungi, especially the Phycomycetes, we still know comparatively little concerning the physiology of sexual reproduction in the Ascomycetes. This is due, perhaps, to the fact that most of the Ascomycetes fail to form their perfect stages in artificial cultures, and that they do not lend themselves as readily as the Phycomycetes to the ordinary technique of physiological experiments in the laboratory.

Klebs (3) in his work with *Eurotium repens* concluded that the quality and the quantity of food exerted no special influence upon the conidia and ascospore formation in this fungus. In case of *Saprolegnia mixta*, however, he (4) found food modifications to be the chief controlling factors in reproduction. Others verified Klebs' findings and extended them to different species. Klebs (5) advanced the theory that ascospore formation in yeasts was determined exclusively by the lack of food at a certain period in the life cycle of these plants. Guillermond (2) summarizing the works of Klebs, Hansen, Saito, etc., concluded that a great number of other factors enter into the physiology of ascospore formation in the yeasts. Raciborski (7) found that high food concentrations hindered zygospore formation in *Basidiobolus*, but Falck (1), on the other hand, reports that high concentrations of a normal nutrient medium induced zygospores in *Sporodinia grandis*. Shear and Wood (8) after reviewing the physiological investigations of other workers concluded that "no cases have been reported in which the evidence was sufficiently conclusive to prove that perithecia were produced in artificial cultures as the direct result of modifications of culture media," and that "the productions of perithecia is a hereditary racial character which does not depend primarily on special conditions of nutriment and

¹ Read at the Boston meeting of the Mycology Section of the Botanical Society of America, December, 1922.

Scientific Paper No. 3, West Virginia Agricultural Experiment Station.

environment." However, we have the work of Smith (9) showing that alkaline media prevented perithecia formation in *Nectriella tracheiphila*. In a former paper the writer (6) reported that perithecial formation in *Valsa leucostoma* was controlled largely by certain chemical factors. Since then the fungus has been studied more extensively, and it has been possible to control its ascigerous and pycnidial reproduction at will.

This fungus seems to possess two strains: in one of these strains the mycelium, the conidia, or the ascospores are capable of giving rise to the perfect as well as to the imperfect stages; in the other one, no matter what the environmental conditions may be, no perithecia develop. However, no morphological differences have been found between the conidial phases of these two strains. Nor could any difference be seen between the physiological efficiency, the nature of infection, and the subsequent pathological symptoms produced by them. The writer feels justified, therefore, in considering the two strains to be identical, with the exception that the ascigerous stage has been lost from the life cycle of one of these, and that probably it can not be introduced again by mere physiological changes. It is also probable that this organism possesses a heterothallic as well as a homothallic strain. While a few organisms of this type have been described, we cannot feel justified in assuming that heterothallism may be widely prevalent in the Ascomycetes, and that it offers the only explanation for the absence of the perfect stage from the life cycle of the Imperfecti. Heterothallism is a fascinating enough subject to become a scientific fad if handled without caution, and it may overshadow the more fundamental facts.

The strain used in following experiments was obtained from an ascospore. The mycelium, conidia, or ascospores of this culture, if grown in a favorable nutrient medium, are capable of producing both the imperfect and the perfect stages. Therefore, all variations pertaining to the control of reproduction, as recorded in this paper, are to be ascribed to environmental modifications rather than to the inherent potentialities of the fungus alone.

THE EXPERIMENTS

Methods. A new technique and a new nutrient solution, which were developed in connection with some other work, have been used throughout these experiments. Filter paper, and small, circular glass capsules of 20 c. c. capacity have replaced agar-agar and test tubes respectively. Filter paper sheets were cut into equal pieces; each piece was moistened with sterile, distilled water, folded and pressed into a circular disc, dried,

placed in the capsule and sterilized in the autoclave. A small quantity (3 to 4 c. c.) of a nutrient solution was then added. The filter paper pads remain constantly moist and afford a good substratum for the growth of the fungus which eventually covers the paper with a mycelial mat and its fruiting bodies. At least thirty different organisms have been grown by the writer on filter paper pads thus prepared, and all have given excellent results. This method enables the investigator to wash his cultures free from one solution, and to transfer them to any desired solution without disturbing the mycelium. The standard solution used in this work consisted of the following ingredients: KH_2PO_4 1.2 grams, MgSO_4 0.6 gram, peptone 0.6 gram, maltose 6.25 grams, malt extract 6.25 grams, distilled water 1000 cc.

The effect of the quantity of food. Three concentrations of the standard solution have been used; no. 1 is the most dilute, containing only 0.37 per cent of food; no. 2 is the standard concentration with 1.5 per cent of food in solution, while no. 3 represents the solution of the highest concentration with a total of 24 per cent of food matter. The following experiments were set up:

1. The fungus was grown on filter paper pads kept saturated with solution no. 1.

2. The fungus was grown on filter paper pads kept saturated with solution no. 2.

3. The fungus was grown on filter paper pads kept saturated with solution no. 3.

4. The fungus was grown on filter paper pads partially submerged in solution no. 2. After the perithecia attained their fullest development, the culture was removed from the dish and a sterile filter paper pad was placed in the old solution and inoculated with the fungus to see if the solution retained enough food to induce another crop of fruit bodies, and whether any of the specific factors affecting either pycnidia or perithecia development were entirely used up by the first crop of fruit bodies.

5. After a good hyphal growth was made in solution 2 and before any fruit bodies appeared, the culture was transferred, at intervals of three days, to a fresh supply of solution no. 2 until the end of the experiment (16 weeks).

Table 1 gives the results: Roman numeral I means very poor, V most abundant, while the other numerals are intermediates between the two.

TABLE 1—*The effect of food concentration upon growth of Valsa leucostoma.*

Experiments	Quantity of mycelium	Quantity of pycnidia	Quantity of perithecia
1. Fungi grown in solution no. 1	I	I	I
2. Fungi grown in solution no. 2	II	II	III
3. Fungi grown in solution no. 3	V	V	V
4. Fungi were grown in solution which had previously matured a full crop of perithecia	I	I	I
5. The cultures were transferred to a fresh supply of the standard solution at intervals of three days.	V	IV	IV

It can be seen that the quantity of mycelium, pycnidia, and perithecia increased in direct proportion with the increase of food concentration; however, the time required for fruiting was found to be proportionately longer in the richer solutions. This is as it should be; a large amount of mycelium formation is a time consuming process, so that a more luxuriant vegetative growth requires a correspondingly longer period for its full development.

It may be assumed that reproduction, as shown by Klebs and others in case of many Phycomycetes and some of the Ascomycetes, is governed by a gradual, or a rapid food reduction, or a total absence of food. If this be the case, then we will be obliged to suppose that when grown in a nutrient solution which contained twenty-four per cent of food, the fungus exhausted all or most of the available nutrient matter before it was able to form perithecia. But this is not the case as we can see from experiment 4. Here a very dilute nutrient solution, after maturing a full crop of perithecia, contained enough food to induce a new mycelial growth and the production of another crop of pycnidia and perithecia. If absence of food, as such, constituted the chief factor in perithecia formation, then we would fail to find this second crop of perithecia. It seems that ordinarily the life cycle of the fungus goes through a mycelium-pycnidia perithecia rotation regardless of the quantity of food, provided that the quality is favorable. A lack of food may hasten reproduction in an otherwise normally nourished mycelium, but it neither induces nor controls it. The mycelium requires certain physiological changes preparatory to reproduction; if food conditions which affect these changes can be controlled, then the regular rotation may be broken up and either the perfect or the imperfect phase of the fungus be induced and perpetuated.

In experiment 5, where the cultures were repeatedly transferred to a fresh supply of nutrient solution, the mycelium, the pycnidia and the

perithecia attained a great increase in their development. A constant presence of food of approximately constant concentration causes a longer period of vegetative growth, delays reproduction, but induces a much more luxuriant fruiting.

Perithecia may be induced not only by a sudden or gradual decrease of food, but also, and much more readily and abundantly, by a sudden increase in the food concentration, as shown in table 2.

The effect of increased or decreased food concentration. Working with twenty different representatives of the Sphaeropsidales,¹ the writer showed that food reduction not only is not a controlling factor in pycnidia formation in most of these organisms, but it even checks reproduction, and that nutrient solutions of high food concentrations are conducive to better vegetative growth and pycnidia formation. The same holds true in case of *Valsa leucostoma* as can be seen in the following table:

TABLE 2—*Effect of increased and decreased food concentration upon Valsa leucostoma.*

Experiments	Quantity of pycnidia	Quantity of perithecia
1. Check; grown in standard solution.	II	III
2. Check; grown in the high concentration of the standard solution.	V	V
3. Cultures were grown in the standard solution; after they made a good vegetative growth, they were washed in sterile distilled water, and transferred to sterile distilled water.	II	III
4.* Cultures were grown in the standard nutrient solution, and after they had made a good vegetative growth, they were washed and transferred to the standard solution of high concentration.	V	I
5. Cultures were grown in the nutrient solution of high concentration, and after they had made a good vegetative growth, they were washed and transferred to sterile distilled water.	II	II
6. Cultures were grown in the nutrient solution of high concentration, and after they had made a good vegetative growth, they were washed and transferred to the solution of standard concentration.	II	V

¹ Paper to appear in American Journal of Botany.

* While perithecia were only very few on the old mycelium, they formed in great numbers on the hyphae which grew out from the edges of the old culture into the solution after the cultures were transferred from the low to the high concentration; no pycnidia appeared on this new growth.

It is evident that while a transfer from low concentration of food to distilled water failed to affect reproduction, a transfer from high concentration to distilled water reduced the quantity of both pycnidia and perithecia. A sudden change of osmotic pressure cannot be considered as a factor, because when the process was reversed, e. g. when the cultures were transferred from low to high concentration the pycnidia formation reached the maximum. Perhaps a gradual leaching of material from the protoplasm into distilled water was responsible for this decrease in reproduction. A transfer from a solution of high food concentration to a greatly dilute solution induced a maximum reproduction of perithecia and suppressed the development of pycnidia; the opposite being the case when the process was reversed. This indicates that the requirements for pycnidia and perithecia formation are not similar and that they depend upon certain physiological changes which do not occur simultaneously but at different times in the growth and age of the mycelium. Why should perithecia formation be preceded by pycnidial development in the normal life cycle of this fungus? Is it because of the decrease of food concentration to a certain minimum as claimed by other investigators in case of some Phycomycetes and as seemingly supported by the experiments 4 and 6 of the foregoing table? In these experiments a sudden increase of food stimulated pycnidia and checked the quantity of perithecia, the opposite being true when the process was reversed. But we have seen that (Table 1, experiments 1 and 4) even in the most dilute solutions the fungus goes through its regular rotation of mycelium-pycnidia-perithecia, and that no apparent suppression of vegetative or reproductive parts occurs. The changes within the mycelium are independent of those in food concentration, and if these physiological changes are timed and subjected to the changes in food concentration, a proper control of the perfect or imperfect phases can be brought about (Table 2, experiments 4 and 6). Why in experiment 4 of table 2, should the old mycelium give rise to only a few perithecia and produce a large quantity of pycnidia, and why should the new mycelium give rise to perithecia and not pycnidia? It seems that physiological changes within the protoplasm are responsible for this. The experiments given in the following pages furnish further proof for the support of this hypothesis.

Control of perithecial and pycnidial formation. Three nutrient solutions have been used in these experiments. Solution A is the standard medium already described; solution B, consists of the standard solution minus malt extract; and solution C, consists of 1 gram of ammonium nitrate,

0.5 gram of dihydrogen potassium phosphate, 0.25 gram of magnesium sulphate, 5 grams of cane sugar, and 100 c. c. of distilled water. When grown in either one of the last two solutions *Valsa leucostoma* fails to produce pycnidia or perithecia, although a fair amount of vegetative growth is made. This fact led to the theory that if a solution unfavorable to reproduction was introduced into the life cycle of this organism, either temporarily or permanently, pycnidia and perithecia development would exhibit interesting modifications. Consequently experiments were set up as shown in table 3.

It can readily be seen that solution C hinders reproduction when it is used by itself or even when it is supplied to the fungus after the cultures have made a good mycelial growth in solution A and then transferred to solution C. However, when the fungus is first grown in solution C and then transferred to solution A, not only no suppression of fruit bodies occurs, but pycnidia are noticeably increased in number. Apparently the unfavorable factor, (ammonium nitrate), which was carried over in the mycelium, was either gradually assimilated, or else it was neutralized by the effect of the more favorable nutrient solution. The increased pycnidial reproduction is to be attributed largely to the fact that the fungus was able to absorb more food from the two different solutions which were introduced into its life cycle, and was also affected by the greater variety of food constituents which were furnished to it. The most remarkable phenomenon, however, is to be observed in the manner of perithecia development; instead of appearing on the mycelium and separate from the pycnidial stromata, as is their natural habit, the perithecia formed on the top of pycnidial stromata, appearing as small, rostrate bodies studded on pycnidial heads.

In the last four experiments of table 3 where the cultures were transferred from solution A to solution C, and then back to solution A again, a marked stimulation of pycnidial reproduction and a total absence of perithecial development is to be noted. It appears that a well developed mycelium, after being transferred to solution C, absorbed too much of the inhibiting factor for perithecia formation, but not enough for the suppression of pycnidia. Perhaps the transfer came at a period in the life of the mycelium when its physiological condition favored pycnidial reproduction rather than perithecial. It should not be assumed that in the last four experiments of the foregoing table the behavior of the mycelium can suggest a possible loss of the power of perithecia formation. A second transfer to a fresh supply of solution A was able to establish the balance so that in due time perithecia appeared, except that they

TABLE 3—*The effect of different solutions upon reproduction of Valsa leucostoma.*

*Experiments	Quantity of pycnidia	Quantity of perithecia	Remarks
1. Check; grown in solution A.	II	III	
2. Check; grown in solution C. <i>Series x</i> ; first grown in solution C and then transferred to solution A.	0	0	Only sterile hyphae.
3. Transferred to solution A after 2½ days	II	III	Pycnidia first to appear; later perithecia formed <i>mostly</i> on top of pycnidial stromata
4. Transferred to solution A after 5 days.	II	III	Same as the preceding.
5. Transferred to solution A after 10 days.	III	III	Perithecia formed <i>only</i> on top of pycnidial stromata
6. Transferred to solution A after 15 days <i>Series y</i> ; first grown in solution A and then transferred to solution C.	III	III	Same as the preceding
7. Transferred to solution C after 2½ days	0	0	Sterile mycelium only.
8. Transferred to solution C after 5 days	0	0	" " "
9. Transferred to solution C after 10 days	0	0	" " "
10. Transferred to solution C after 15 days <i>Series z</i> : first grown in solution A till a good hyphal growth was obtained, then transferred to solution C, and 2½, 5, 10, and 15 days later transferred to solution A.	0	0	" " "
11. Transferred 2½ days later.	III	0	In series z when the cultures were again washed in sterile distilled water, and were transferred to a fresh supply of solution A, perithecia appeared in due time.
12. Transferred 5 days later.	IV	0	
13. Transferred 10 days later.	IV	0	
14. Transferred 15 days later.	IV	0	

* Before each transfer the cultures were thoroughly washed in sterile distilled water.

altered their usual habit and formed directly on the top of pycnidial stromata. The hyphae of the old mycelium and the hyphae of the stromata have different ages and different physiological potentialities, and it is only natural for them to exhibit different reactions towards the environmental factors. This changed location of fruiting bodies is further shown in table 4.

TABLE 4—*Changed location of perithecia and pycnidia of Valsa leucostoma due to changed food factors.*

Experiments	Quantity of pycnidia	Quantity of perithecia	Remarks
1. The fungus was grown in solution A; after the perithecia were fully developed the cultures were washed and transferred to a fresh supply of solution A.	0	III	Perithecia gave rise to tufts of hyphae at their tips. These tufts developed into stromata, and without giving rise to pycnidia, produced perithecia directly.
2. The fungus was grown in solution A; after the perithecia were fully developed the cultures were washed and transferred to solution C; seven days later they were again washed in distilled sterile water and were transferred to a fresh supply of solution A	III	III	Perithecia gave rise to pycnidial stromata, and these eventually produced perithecia, thus forming two layers of perithecia separated by a layer of pycnidia.

In the first experiment pycnidia were eliminated entirely because physiological changes which occurred in the mycelium and gave rise to the perfect stage persisted when the entire culture was transferred intact and was disturbed but very little. When the mycelium is cut to pieces, the physiological balance is disturbed and it becomes necessary for the fungus to go through the mycelium-pycnidia-perithecia rotation again. Similarly, if we examine experiment 4 in table 2, we shall find further support for this theory. In that case when the cultures were transferred from the low food concentration to the high food concentration, the old mycelium, in which physiological changes favored only pycnidial reproduction, yielded a scanty crop of perithecia, while the hyphae which formed after the transfer, developed a large quantity of perithecia and no pycnidia.

Experiment 2 of table 4 shows that the location and the relationship of the perfect and the imperfect fruiting bodies to each other can be changed at will by the rotation and the nature of the nutrient solutions.

The effect of the different food constituents. The influence of dihydrogen potassium phosphate, magnesium sulphate, peptone, and maltose, singly and in all possible combinations, was tested. Malt extract was reserved for a separate experiment.

The fungus was grown in the standard solution; when a good mycelial growth was obtained, and before any fruiting bodies formed, the cultures were washed and transferred to the solutions listed in the following table. The amounts of the ingredients used in these solutions are the same as given in the formula of the standard solution.

TABLE 5—*The effect of different food constituents upon Valsa leucostoma.*

Experiment no.	Quantity of pycnidia	Quantity of perithecia	Remarks
1. Transferred to distilled water	II	III	
2. Transferred to a fresh solution of the standard medium	III	IV	
3. Transferred to KH_2PO_4 , MgSO_4 , peptone, maltose	II	IV	
4. Transferred to MgSO_4 , maltose, peptone	III	III	
5. Transferred to KH_2PO_4 , maltose, peptone	III	III	
6. Transferred to MgSO_4 , KH_2PO_4 , peptone	III	III	
7. Transferred to KH_2PO_4 , peptone	III	III	Perithecia formed on top of pycnidial stromata only.
8. Transferred to KH_2PO_4 , MgSO_4	II	III	
9. Transferred to MgSO_4 , peptone	III	III	Perithecia formed on top of pycnidial stromata only.
10. Transferred to maltose, peptone	III	IV	
11. Transferred to maltose, MgSO_4	III III	III III	Perithecia formed on top of pycnidial stromata only.
12. Transferred to KH_2PO_4	II	II	Perithecia formed on top of pycnidial stromata only.
13. Transferred to MgSO_4	II	III	
14. Transferred to peptone	II	II	Perithecia formed on top of pycnidial stromata only.

Table 5 shows that all of the food constituents of the standard nutrient solution are able to induce both the perfect and the imperfect fruiting bodies when used either by themselves or in all possible combinations. Some seem to be a little more favorable than others. Dihydrogen

potassium phosphate or peptone, when used singly, proved to be the least favorable but when combined, they induced a better reproduction.

The effect of sugars. Four sugars in different concentrations have been used in these experiments. Of these sugars, maltose, glucose and levulose were pure, while cane sugar was the commercial granulated product. The fungus was grown in the standard nutrient solution; after it made a good hyphal growth and before any of the fruiting bodies appeared, the cultures were washed in distilled sterile water and were transferred to the various sugar solutions listed in table 6.

TABLE 6—*The effect of nitrates upon Valsa leucostoma.*

Experiment No.	Quantity of pycnidia	Quantity of perithecia
1. M/8 maltose	I	0
2. M/16 "	II	0
3. M/32 "	II	1
4. M/64 "	II	III
5. M/8 cane sugar	0	0
6. M/16 " "	I	0
7. M/32 " "	II	0
8. M/64 " "	II	III
9. M/8 Glucose	I	0
10. M/16 "	II	III
11. M/32 "	II	III
12. M/64 "	II	III
13. M/8 levulose	I	0
14. M/16 "	I	III
15. M/32 "	I	IV
16. M/64 "	I	IV

Higher concentrations of sugars tend to reduce pycnidia formation and hinder perithecia development. M/8 concentration of cane sugar solution prevented reproduction altogether. However, it can be seen that the range of pycnidial formation is much wider than that for perithecia, and in this respect *Valsa leucostoma* behaves like most of the other fungi which have been worked with by other investigators. Levulose seems to be the best sugar for inducing the perfect stage, and is the least favorable for pycnidial development.

The effect of nitrates. After the fungus made a good hyphal growth in the standard solution, and before the first fruit bodies appeared, the cultures were washed and transferred to the different solutions of nitrates as listed in following table.

TABLE 7—*The effect of sugars upon Valsa leucostoma.*

Experiment no.	Quantity of pycnidia	Quantity of perithecia
1. M/8 ammonium nitrate	0	0
2. M/16 " "	0	0
3. M/32 " "	0	0
4. M/64 " "	I	0
5. M/8 calcium nitrate	III	0
6. M/16 " "	II	0
7. M/32 " "	II	0
8. M/64 " "	II	0
9. M/8 sodium nitrate	0	0
10. M/16 " "	II	0
11. M/32 " "	II	0
12. M/64 " "	II	0
13. M/8 potassium nitrate	III	0
14. M/16 " "	III	0
15. M/32 " "	II	0
16. M/64 " "	II	0

Nitrates, as we can see, hinder perithecial development altogether. Ammonium nitrate is uniformly unfavorable for reproduction, and in only the most dilute solution was it able to induce just two or three single pycnidia without stroma. Calcium nitrate and potassium nitrate seem to be more favorable to pycnidia formation in higher concentrations than sodium nitrate.

The minimum absorption of malt extract necessary for reproduction. It was found that the fungus would not reproduce in the ordinary nutrient solution if malt extract was left out from this medium. The solution with malt extract (the standard) has been termed solution A; the one without malt extract is termed solution B for convenience. It was proposed to find out if a mycelium grown in solution B, then transferred to 0.625 per cent of malt extract solution for various lengths of time, and finally transferred back to a fresh supply of solution B would absorb a sufficient quantity of malt extract to induce pycnidia and perithecia. A number of cultures were made in solution B; after a good hyphal growth was obtained the cultures were washed and transferred to malt extract solution for one, five, ten, fifteen, thirty minutes, for one, two, four, eight, twelve, twenty-four, forty-eight, and seventy-two hours, then washed in four changes of distilled sterile water and transferred to a fresh supply of solution B. Table 8 gives the results.

Ten minutes in malt extract solution appears to be the approximately minimum length of time for pycnidial reproduction, and fifteen minutes

TABLE 8—*The minimum absorption of malt extract necessary for reproduction of Valsa leucostoma.*

Experiments	Quantity of pycnidia	Quantity of perithecia
1. Check; cultures were washed and transferred to a fresh supply of solution B without transferring to malt extract solution.	0	0
2. Check; cultures transferred to malt extract solution and left there	II	III
3. Cultures were kept in malt extract solution 1 minute	0	0
4. Kept in malt extract 5 minutes	0	0
5. Kept in malt extract 10 minutes	I	0
6. Kept in malt extract 15 minutes	I	I
7. Kept in malt extract 30 minutes	I	I
8. Kept in malt extract 1 hour	I	I
9. Kept in malt extract 2 hours	II	II
10. Kept in malt extract 4 hours	II	II
11. Kept in malt extract 8 hours	II	II
12. Kept in malt extract 12 hours	II	II
13. Kept in malt extract 24 hours	II	II
14. Kept in malt extract 48 hours	II	II
15. Kept in malt extract 72 hours	II	III

for the perithecial development. Longer periods induce better reproduction, but it seems that after a certain period, (72 hours), malt extract does no longer exert any stimulative influence upon reproduction, as a comparison of check no. 2 and experiment 15 will show. It should be remembered in this connection that a mycelium well nourished in the standard nutrient solution reproduces just as well whether it is kept in the same solution or whether it is washed and transferred to distilled water. It seems that after a certain period of growth in a dilute nutrient solution the mycelium no longer depends on the outside food for the formation of its fruit bodies, but utilizes the food and the energy stored within its own protoplasm. There is a balance between the fungus and its food; at a certain stage a withdrawal of food tends to weaken the mycelium, while at other stages it exerts no appreciable influence. This is not because of a total absence of food in the old nutrient solution, or because of a possible presence of some toxic substance given off by the growing fungus, for we have seen that the nutrient solution, after giving rise to one crop of fruit bodies, was able to develop a second crop. Nor can we say that the mycelium, after reaching a certain stage of growth, is unable to absorb and utilize the food in the nutrient solution, for we have seen that when the cultures, after the formation of fruit bodies, were transferred to a fresh supply of the nutrient solution, were

able to absorb food, grow, and give rise to new fruit bodies. In case of higher concentrations of food, however, a constant presence of the nutrient solution seems to be essential. We have seen that cultures after making a good growth in a higher concentration of nutrient solution failed to do well when transferred to distilled sterile water, while a maximum reproduction was the result when they were transferred to a very dilute medium. A sudden increase of food during any part in the growing life of the fungus is conducive to an infinitely better reproduction, and its sudden withdrawal marks a sharp reduction in the quantity of fruit bodies. We can make no definite rules and mould the properties of the living protoplasm into standard forms, because an organism exhibits different reactions with each change occurring either within or without its protoplasm, and these changes are not, ordinarily, perceptible enough to be interpreted according to any set standards.

SUMMARY

1. The strain of fungus used in these experiments produces pycnidia and perithecia if grown in a favorable nutrient medium. Conidia or ascospores show identical physiological reactions to the various solutions used.

2. The quantity of mycelium, pycnidia, and perithecia increased in direct proportion to the increase of food concentration.

3. The same solution, after maturing a full crop of fruit bodies, had a sufficient amount of food left to develop a second crop.

4. When the food supply of the fungus was renewed every three days for about sixteen weeks, mycelium, pycnidia, and perithecia formed in great abundance, showing that an absence or a decrease of food was not a factor in the reproduction of this organism.

5. When transferred from the standard food solution to distilled water, there was no appreciable difference in the quantity of fruit bodies formed; when, however, the mycelium was transferred from a solution of high concentration to distilled water, there was a sharp decrease in reproduction.

6. When the mycelium of this fungus was transferred from the standard solution to the high concentration of the same solution, pycnidia increased in number while perithecia decreased; when the process was reversed, the results were also reversed.

7. Solution C, where cane sugar furnished the carbohydrates and ammonium nitrate supplied the nitrogen, was unable to induce either pycnidia or perithecia. When, however, the mycelium was grown in

this solution and then transferred to the standard solution, there was a marked increase in reproduction. When the process was reversed, e. g. the mycelium was transferred from the standard solution to solution C., reproduction was suppressed. When the mycelium was grown in the standard nutrient solution, then washed and transferred to solution C, and a few days later transferred back to the standard solution again, pycnidia formed in great abundance, while perithecia were suppressed.

8. When the fungus was grown in the standard solution until the perithecia formed, and then washed and transferred to a fresh supply of the nutrient solution, perithecia gave rise, at their tips, to a new crop of perithecia, but no pycnidia developed.

9. When the fungus was grown in the standard solution until the perithecia formed, and then it was washed and transferred to solution C, and a few days later it was again washed and transferred to a fresh supply of the standard solution, perithecia gave rise, at their tips, to stromata with mature pycnidia; eventually these pycnidial stromata gave rise to perithecia. The perfect fruiting bodies appeared as small, rostrate structures studded on globose stromata.

10. When the fungus was allowed to form a good crop of mycelium and then it was washed and transferred to the different food constituents of the standard solution (these ingredients being used either singly or in all possible combination), no suppression of either type of reproduction took place.

11. When the mycelium of the fungus was developed in the standard solution, and then washed and transferred to M/8, M/16, M/32, and M/64 solutions of maltose, cane sugar, glucose, and levulose, it was found that the high concentrations checked perithecia formation; M/8, and M/16 concentrations of maltose, M/8, M/16, M/32 concentrations of cane sugar, and M/8 concentration of glucose and levulose hindered perithecia formation, while only the M/8 concentration of cane sugar prevented pycnidial formation. Levulose was found to be the most favorable for perithecia development and the least favorable for pycnidia production.

12. When the mycelium of the fungus was developed in the standard solution, and then washed and transferred to M/8, M/16, M/32, and M/64 concentrations of ammonium nitrate, calcium nitrate, sodium nitrate, and potassium nitrate solutions, perithecia formation was hindered altogether. M/8, M/16, and M/32 concentrations of ammonium nitrate, and M/8 concentration of sodium nitrate prevented pycnidia formation as well.

13. Solution B, which contains no malt extract but otherwise is just like the standard solution, failed to induce reproduction. When the fungus was grown in this solution until a good mycelial growth was obtained, then washed and transferred to 0.6 per cent of malt extract for one, five, ten, fifteen, and thirty minutes, and one, two, four, eight, twelve, twenty-four, forty-eight, and seventy-two hours, and then washed and transferred to a fresh supply of solution B, it was found that one and five minutes' exposure to malt extract solution induced no reproduction whatsoever; ten minutes' exposure induced pycnidia, and fifteen minutes' exposure induced both pycnidia and perithecia. Longer exposures merely served to increase the number of fruit bodies.

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ON THE RELATION OF TEMPERATURE TO THE DAMPING-OFF OF GARDEN-CRESS SEEDLINGS BY PYTHIUM DEBARYANUM AND CORTICIUM VAGUM

TAKESUO HEMMI

WITH TWO FIGURES IN THE TEXT.

INTRODUCTION

The economic importance of damping-off diseases in the United States was first emphasized by Atkinson (1), who stated that treatment of these diseases requires special attention to the environmental conditions that influence the development of the pathogenic organisms. He concluded that damping-off is favored by high temperature, wet soil, humid atmosphere, weak light and lack of ventilation. Rhizoctonia (the sterile stage of *Corticium vagum* B. et C. var. *solani* Burt) causes not only damping-off of seedlings, but also cankering of potato stems. Rolfs (17, 18) concluded that high temperature and abundant moisture were accompanied by rapid development of this organism on potato in Colorado. Balls (2) observed that high soil temperature apparently prevented the injury of cotton by Rhizoctonia, while lower soil temperatures favored the pathogenic action of this fungus. The same writer (3) found experimentally that Rhizoctonia mycelium infected cotton seedlings at 20° C., but not at 33° C. Morse and Shapovolov (14) observed great injury of field potato plants by Rhizoctonia in the hottest part of the summer, and Peltier (15), who studied various strains of this fungus experimentally, expresses the opinion that high temperature, together with too little or too much moisture, favors injurious infection. Johnson (6) showed that excessively moist and hot weather favors the occurrence of damping-off diseases. He considered the optimum temperature for the growth of Rhizoctonia to be about 25° C., while that of Pythium was thought to be about 33° C., and he pointed out the need for a knowledge of the relation of temperature to the occurrence of these diseases.

Gilman (5) and Tisdale (19, 20) studied first the relation of soil temperature to Fusarium infections. Jones (10) emphasized the desirability for studying environmental relations in plant pathology, dwelling especially on the great need for studies of soil temperature as related to the pathogenicity of the organisms that cause plant diseases. Johnson

and Hartman (8) studied the relation of soil conditions to rootrot of tobacco, reaching the conclusion that soil temperature is undoubtedly the most important of these conditions. Clayton (4) and several other authors reported experimental results from studies on several diseases, and Jones (11) has recently summarized the progress thus far made in this important field. Johnson (7) further gave special attention to air temperature as an influential condition.

Although practical experience seems to teach that damping-off by *Pythium* is more serious with high temperatures and that caused by *Rhizoctonia* is more serious with lower temperatures, this relation has not been thoroughly studied experimentally. One report of experimentation of the sort just indicated has been published by Richards (16), on the pathogenicity of *Rhizoctonia* to potato stems, as related to soil temperature. Pure cultures or sclerotia of the fungus produced lesions near the base of the stem with temperatures of 9° to 27° C., the greatest damage occurring between 15° and 21° C. The fungus was pathogenically most active at 18° C. Richards concluded that it is only with temperatures below 24° C. that this fungus is a serious hindrance to potato production.

The present paper reports the results of an experimental study of the relation of soil temperature and air temperature to the pathogenicity of *Pythium DeBaryanum* Hesse and of *Corticium vagum* B. et C. var. *solani* Burt towards seeds and seedlings of garden cress (*Lepidium sativum* L.) in greenhouse cultures. The experiments were done in the Department of Plant Pathology of the University of Wisconsin, during the spring of 1922. The writer wishes to express his indebtedness to Doctors James Johnson, A. G. Johnson and F. R. Jones, for suggesting this study, and his special gratitude to Doctors James Johnson and Jones, for kindly supplying cultured strains of the fungi and for much other help. The writer is also greatly indebted to Doctors L. R. Jones, G. W. Keitt, E. M. Gilbert, and others, for helpful suggestions. Finally, he wishes to express his hearty thanks to Doctor Burton E. Livingston, of the Laboratory of Plant Physiology of the Johns Hopkins University, who read the original draft of the manuscript and did much editorial work on it, especially with regard to its condensation into as brief a form as possible.

THE ORGANISMS USED

From seven strains of *Pythium* and an equal number of *Rhizoctonia*, a single strain of each was selected by means of preliminary tests on pathogenicity toward seedlings of garden cress and of lettuce. The

Pythium strain selected had been isolated from sugar beet by Dr. F. R. Jones, and the Rhizoctonia strain had been secured from a soil in Connecticut by Dr. James Johnson. Pure cultures of each of these were prepared and were used as sources for inoculation in the experiments to be considered below, the Pythium being grown on corn-meal agar and the Rhizoctonia on potato agar. Besides exhibiting pathogenicity toward garden cress, both of these strains produced disease in tomato and cucumber seedlings. Lettuce seedlings were seriously injured by this Rhizoctonia strain, but apparently not by this Pythium strain.

EXPERIMENTATION WITH SOIL TEMPERATURE ALONE APPROXIMATELY MAINTAINED

In the first series of experiments only the soil temperature was controlled. The culture vessels were galvanized sheet-iron cylinders, 15 cm. in diameter and 25 cm. deep, standing in water baths with suitably controlled temperatures. The apparatus used has been described by Jones (10) and by Johnson and Hartman (8). The cylinders were nearly filled with soil, and the soil surface was affected by the air temperature of the greenhouse. Consequently the surface soil varied in temperature somewhat, tending to follow the fluctuating air temperature. Attempts to reduce the temperature variation in the superficial soil layers, by means of paper covers, etc., were not very successful. The upper layers of soil in the different baths were very different, however, in spite of this variation.

A 5-cm. layer of artificially infested soil (made by mixing pure cultures of the fungus with sterilized soil) was added above an 11 cm. layer of sterilized soil in the bottom of each cylinder, and a 2.5 cm. layer of sterilized soil was added above this, on which 100 or 150 cress seeds were placed, covered with another layer of sterilized soil about 1.5 cm. deep. Controls were prepared in the same manner, but with sterilized soil only. Soil temperatures for the superficial layer were determined from time to time, by means of thermometers, and water was added when it seemed to be needed.

After the seedlings had appeared they were frequently examined, to determine the degree of infection for each temperature tested. At the end of each experiment the number of healthy seedlings in each control cylinder and the number in the corresponding infested cylinder were recorded. In many cases the seeds were infected and failed to germinate, or the seedlings were killed before reaching the soil surface, and an in-

direct method of determining the degree of infection was consequently employed. To secure a measure or index of infection, the number of healthy seedlings in the infected cylinder was expressed as a percentage of the corresponding number in the control cylinder at the same temperature, and this value was finally subtracted from one hundred. The temperature ranges secured in the upper soil layers were about as follows, from four to six of the ranges being tested for each fungus in each experiment:

13-17° C.	18-22° C.	23-25° C.	28-30° C.
14-15° C.	18-23° C.	23-27° C.	28-31° C.
14-16° C.	19-21° C.	24-27° C.	28-32° C.
14-17° C.	19-23° C.	25-27° C.	29-30° C.
15-18° C.	21-24° C.	25-28° C.	29-31° C.
16-17° C.	22-24° C.	26-28° C.	29-33° C.
16-18° C.	22-25° C.	26-29° C.	30-32° C.
18-21° C.	22-27° C.	27-30° C.	32-34° C.

After the completion of six experiments, during the carrying out of which a number of difficulties in technique were encountered and wholly or partially overcome, it was found that the consensus of all the results was as follows: With low and with high temperature ranges, and for both the fungi, infection was not as serious as with medium ranges. Neither fungus produced infection with the highest range tested (32-34° C.). In one case *Rhizoctonia* produced infection with temperature 30-32° C. With temperature 29-33° C. both fungi failed to give infection. With temperatures above 30° it is, however, unlikely that infection by this strain of *Rhizoctonia* would appear under the general conditions of these experiments, while infection by this strain of *Pythium* should be expected frequently. With lower temperature ranges there was always more infection by both the fungi. The controls were uniformly without infection. Damping off by *Pythium* was generally most serious with temperature ranges between 22° and 27°, while that by *Rhizoctonia* was generally most serious with temperature ranges between 16° and 23°. With the lowest temperature tested both fungi were still dangerous parasites. From counts of the seedlings in each control cylinder it appeared that the highest percentage of germination of the seeds occurred within the temperature range 18-23° C. Germination was markedly retarded with temperatures above 30° C.

These generalizations are not to be considered as precise; they are only rough approximations. Whether or not infection is to occur in any culture is of course determined by a group of conditions, of which the soil temperature and the presence of the active fungus are only two.

Evidence was noted supporting the supposition that air temperature and air humidity are additional influential conditions in this respect. The writer has also to call attention to the fact that, with both fungi, growth often occurs on the surface of the soil and the seedlings are attached at that level. Another series of experiments will now be considered, for which the soil and the air temperatures were both controlled.

EXPERIMENTATION WITH BOTH SOIL AND AIR TEMPERATURES MAINTAINED AND ALIKE

The experiments to be dealt with now were carried out with the culture vessels (4-inch pots of the ordinary type used in greenhouses) in thermostatically controlled chambers, the temperature of the soil being very nearly the same, in every case, as that of the air above, and both soil and air temperatures were maintained within a rather narrow range. The chambers used have been described by Johnson (7). Aside from the form of culture vessel used, and the temperature and air-humidity control, the procedure was essentially similar to that followed in the experiments considered above. The water-vapor-tension deficit¹ was nearly the same for all temperature ranges. For the entire range of mean temperatures considered (10°–37° C.), the deficit range was from 3.5 mm. to 9.1 mm. of mercury column. Each experiment included, for each temperature range, six cultures, two for each of the two fungus strains and two as controls.

Four successful experiments were carried out. The numerical data for these summarized in table 1. The temperature and relative humidity ranges are given in the first two columns the approximate means of temperature and of relative humidity are given in the next two columns, and the approximate mean saturation deficits are given in the fifth column. The number in parenthesis in the first column denotes the serial number of the experiment in which the given temperature range was tested.

¹ The vapor-tension deficit is a measure of the tendency of the air to take up water vapor, disregarding wind movements, which may be assumed to have been about alike in all chambers. It is derived from the mean temperature and the mean relative humidity in each case, by multiplying the maximum vapor pressure of water for the given temperature by the relative humidity value (considered as hundredths) and then subtracting the product from the maximum vapor-pressure value. Thus, for an mean temperature of 10 C. and a mean relative humidity of 62 per cent., we multiply 9.165 mm. by .62 and obtain 5.682 mm., which we subtract from 9.165 mm., obtaining 3.483 mm., the deficit value sought. The maximum vapor tension is of course to be found from tables of physical constants. In this connection, see (9, 12, 13).

The calculated percentages of infection are given, for each of the two fungi, in the sixth and seventh columns. Percentages of infection that are above 80 are shown in bold-face type in the table. Percentages of germination (number of seedlings produced in the controls, divided by 80 (number of seeds planted), and that quotient multiplied by 100 are shown in the last column.

TABLE 1.—Numerical data from experiments with soil temperature and air temperature maintained and alike.

Temperature Range	Relative Humidity Range	Mean Temperature (approx.)	Mean Relative Humidity (approx)	Mean Vapor-tension Deficit (approx)	Percentage of Infection by		Percentage of Seed Germination (controls)
					<i>Pythium</i>	<i>Corticium</i> (<i>Rhizoctonia</i>)	
deg. C.	per cent	deg. C.	per cent	mm. of Hg			
8-12 (2)	54-70	10	62	3.5	24 1	72.4	72.5
13-18 (1)	44-55	16	50	6.7	45 6	87 7	71.3
15-18 (3)	40-60	17	50	7.2	64.4	93 2	73.8
19-21 (4)	40-60	20	55	7 8	92.7	69 1	68.8
20-23 (2)	54-72	22	63	7 3	83.6	85 2	76 3
22-26 (3)	60-80	24	70	6.6	85.5	80 0	68.8
25-27 (3)	68-83	26	76	6.0	96 2	67 9	66 3
28-31 (2)	75-93	30	84	5 0	87 5	68.8	40 0
28-32 (1)	68-85	30	77	7.3	90.0	53 3	37 5
33-35 (4)	65-88	34	77	9.1	0	0	0
35-38 (1)	80-90	37	85	7.0	0	0	0

The following paragraphs bring out the more striking points of each experiment.

Experiment I. March 18-31. With temperature range 35-38°, germination was almost inhibited in both infested and control cultures, and germination was best with the lowest range (13-18°). The plantlets are shown to have been much more seriously attacked by *Pythium* with temperature range 28-32° than with range 13-18°. On the other hand, *Rhizoctonia* injury was markedly more serious with range 13-18° than with range 28-32°.

Experiment II. April 4-22.—Germination is shown to have been best with temperature range 20-23°, but with range 8-12° the germination percentage is nearly as high. The most serious injury by *Pythium* occurred with range 28-31°, while *Rhizoctonia* injury was most serious

with range 20–23°. With range 8–12° one seedling was affected by *Rhizoctonia* after its shoot emerged from the soil. At the end of the experiment all cultures were removed from the chambers and placed on the greenhouse bench, where the temperature of the surrounding air might be considered as suitable for infection. After two days marked infection was shown in all infested cultures, but the controls remained free from disease.

Experiment III. April 25–May 7.—Germination was nearly the same in all the controls. The most serious *Pythium* injury occurred for temperature range 25–27°, while *Rhizoctonia* injury was most marked for range 15–18°. Range 22–26° gave intermediate infection percentages for both fungi.

Experiment IV. May 18–28.—For temperature range 33–35°, no germination occurred in control cultures (two seedlings grew in one infested pot), but germination was good for the other range of this experiment (19–21°). Both fungi attacked the plants vigorously with the lower range.

Considering all the data of table 1, it appears that the *Pythium* strain here dealt with caused disease in more than 80 per cent of the seeds and seedlings for temperature ranges from 19–21° to 28–32°, or from about 20° to about 30°, and all ranges thus included were apparently about alike in this respect. With lower temperature ranges the percentage of infection was progressively lower, but the lowest range tested (8–12°) gave 24.1 per cent of injury. For the highest two ranges tested, there was no germination in any control pots (few seedlings came up in some infested pots), and it appears that a temperature high enough to prevent infection by this *Pythium* would be so high as to inhibit seed germination of this *Lepidium*.

As to infection by the *Rhizoctonia* strain here studied, the infection percentages are above 80 per cent for ranges 13–18°, 15–18°, 20–23°, and 22–26°. Disregarding the exceptional case of range 19–21°, we may say that this fungus was very injurious for temperatures from about 16° to about 24°. The lowest range tested (8–12°) gave an injury value of 72.4 per cent. For ranges above 24° the injury was smaller, but its value is 53.3 per cent, for the highest range with which germination occurred in the controls (28–32°).

These results indicate clearly that the range of temperatures favoring injury to these seedlings is very wide for both fungi but that this *Pythium* was most injurious for temperatures between 20° and 30°, while this *Rhizoctonia* was most injurious for a lower range between 16° and 24°. (See figs. 1, 2.)

The relation of temperature to seed germination in these experiments may be studied by means of the data in the last column of the table. Germination seems to have been sensibly about alike (68.8–76.3 per cent) for all temperatures from about 10° to about 24°. With higher temperatures germination was hindered, and for ranges 33–35° and 35–38° it was inhibited.

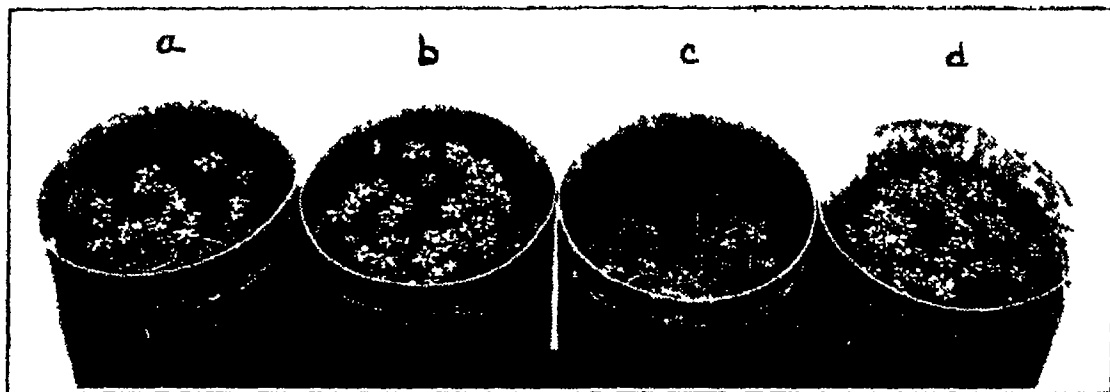


Fig. 1. Seedlings of *Lepidium sativum* L. grown with soil temperature about 26° C. a, in *Corticium*- (*Rhizoctonia*) infested soil. c, in *Pythium*-infested soil. b and d, in uninfested soil.

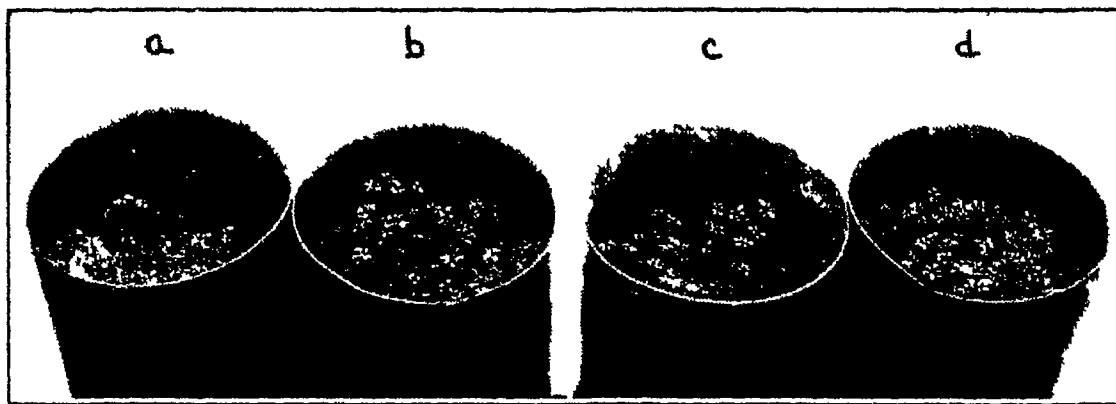


Fig. 2. Seedlings of *Lepidium sativum* L. grown with soil temperature about 16° C. a, in *Corticium*-(*Rhizoctonia*) infested soil. c, in *Pythium*-infested soil. b and d, in uninfested soil.

GENERAL CONCLUSIONS

In a general way, the results of the two series of experiments considered above agree in showing (1) that both fungi were injurious to garden cress seeds and seedlings for temperatures between about 10°

and about 30° C. No statement for higher temperatures can be made, since those tested inhibited seed germination. Lower temperatures were not tested. (2) These results also show that the *Pythium* strain here used was less injurious to these *Lepidium* seedlings than was the *Rhizoctonia* strain for temperatures below about 20° C., and more injurious than the *Rhizoctonia* strain for temperatures above about 24° C. (3) For temperatures from about 17° C. to about 26° C., both fungi were very injurious. (4) The temperature ranges of most active infection may be stated, approximately, as follows:—

<i>Pythium de Baryanum</i>	20–30°C.
<i>Corticium vagum</i> (<i>Rhizoctonia</i>)	16–24°C.

(5) The *Lepidium* seeds used germinated well for temperatures from about 10° to 24° C. Lower temperatures were not tested. Germination was markedly retarded or entirely inhibited with temperatures above about 30° C.

These conclusions are of course not to be taken as applicable to other fungi or hosts and they are only approximate, even for the organisms employed in these experiments and for these experimental conditions.

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PHYTOPATHOLOGICAL NOTES

Blister rust of the white pine. The question as to whether *Cronartium ribicola* would winter over on the currant has been before American plant pathologists almost ever since the white pine blister rust became an important factor on America. Thus far no satisfactory experiments have been carried out which would give a positive answer to this question. In 1921 a planting of black currants near Blue Anchor (Camden county) N. J. was selected for field test on this problem. This planting has been known to be infected for several years and although the surrounding country has been thoroughly worked by both Federal and State scouts the nearest known alternate hosts were fully three quarters of a mile distant and were not infected. In fact so far as known there are no infected pines in South Jersey. The planting is an inter-row crop in a young apple orchard which is just coming into bearing and is bounded on two opposite sides by narrow strips of forest. There is considerable forest in the immediate vicinity but no large areas.

This planting was examined in the late summer of 1921 by Dr. Perley Spaulding of the U. S. Bureau of Plant Industry and the writer, and found to be heavily infected with *C. ribicola* and it was definitely decided to make a field test in 1922. Therefore, in March 1922 a double thickness cheese cloth tent was erected over a considerable number of plants by County Farm Demonstrator S. F. Foster and the writer. The cloth was furnished by the U. S. Bureau of Plant Industry. The tent was stretched over a ridge pole and securely anchored to the ground. During the summer of 1922 the entire planting was inspected by Dr. Haven Metcalf of the U. S. Bureau of Plant Industry and the writer in May, by Dr. H. H. York and the writer in June and by the writer alone in August. At the time of the first inspection a single infected leaf was found at considerable distance from the tent. At the time of the second inspection a considerable amount of infection was found in the planting. At the time of the third inspection the plants under the tent were found to be very generally infected but the infection was very light, i. e. there was rarely more than one or two spots on any one leaf. Infected plants were found throughout the planting but the infection was very light as compared with 1921. The writer realizes that the evidence is not conclusive, that it is possible that spores may have sifted through the cheese cloth tent but the evidence indicates that the fungus may possibly be carried from year to year on the currants.—MEL T. COOK.

Dwarf asparagus. At the Toronto meeting of the American Phytopathological Society, the writer reported a disease of asparagus which was of occasional occurrence throughout the state of New Jersey. From time to time for several years, dwarfed or stunted asparagus has been sent in for examination during the cutting season. These specimens were short, usually thick and slightly irregular in shape. They usually showed a slight cracking on the part of the stem below the surface of the ground and a discoloration varying from a slight brown to a pronounced rust. The most marked character was the dwarfing and it was this point that brought out the inquiries. In all cases, more or less *Fusarium* was found on the specimens. The complaints were so few that no efforts were made to study this trouble until the summer of 1921 when the writer's attention was called to two cases where the plants over circular areas in the fields were dwarfed and apparently dying. The disease was most severe in the centers of the areas, gradually becoming less prominent towards the margins which were not sharply defined. The areas had very much the appearance of having been struck by lightning but this idea was abandoned, (1) because the plants in the areas were never equal at any time to the surroundings plants although the variations were least noticeable very early in the season, (2) because there was no wilting, (3) because no other plants were injured, (4) because the trouble occurred over this same area from year to year. In these cases the diseased plants showed a pronounced brown or rusty discoloration on the stems below the surface of the ground, which resembled the browning on potato stems when infected with *Rhizoctonia*. The plants were dwarfed and died very slowly but new shoots were sent up and usually became infected. These plants were found to be heavily infected with *Fusarium* sp. During the winter of 1921-22 a number of healthy plants were grown in the green house and were inoculated with the organism from time to time. The results of these studies are as follows: (1) the organism is a pathogen producing the symptoms described above, (2) the younger the shoot the more easily it is infected, (3) when the stem gets above the ground without infection it is not likely to become infected, (4) infection is most easily accomplished if the stems are slightly injured, (5) very young stems may be infected without previous injury, (6) stems four inches or more in height are not likely to become infected, even though injured. Checks were used with every inoculation and the organism was recovered from the diseased plants.—MEL T. COOK.

A disease of dahlias. During the month of September 1922 Dr. M. A. Howe of the New York Botanical Garden called the attention of the writer to a mild disease of dahlias. This disease occurred on only two varieties, Snowdrift and Shasta, both of which are white. The first evidence of the disease is a water-soaked appearance of the stems accompanied by a wilting of the infected parts. The diseased parts die and become black and dry. The central cavities of the stems are more or less completely filled with a luxuriant growth of mycelium and a considerable number of large sclerotia developed. The sclerotia are somewhat larger and more abundant in Snowdrift than in Shasta and this holds true in the cultures from them. This organism has been determined by Professor H. H. Whetzel as *Sclerotinia libertiana*. The disease is not severe and cannot be considered of major importance. The writer is unable to say at this time whether this is a weak strain of the fungus or whether the plants are resistant. No experiments have been tried.—MEL T. COOK.

White pine blister rust in Michigan. Four different pine infections were located in Michigan during the past season. Three of these were found in Oakland County in the vicinity of Birmingham, and Royal Oak. These outbreaks clearly demonstrate the persistence of the rust in localities where it has previously appeared. In the vicinity of Birmingham, local currants and gooseberries were infected. The center of the infection was discovered early in the season and the diseased plants destroyed. *Ribes aureum* Pursh., *Ribes americanum* Mill., *Ribes cynosbati* L., *Ribes nigrum* L., and cultivated red and white currants (*Ribes vulgare* Lam.) were infected. *Ribes americanum* led the list in the number of diseased bushes. This was probably due to its abundance and favorable location with respect to the diseased pine. The dry 1922 season in this section of the state is also believed to have materially reduced the number of infections. The fourth pine infection was discovered about seven miles east of Grand Rapids in Kent County. This tree was found in a planting made from stock received from Ussy, France, in 1908 before the establishment of quarantine restrictions. This is the first time the rust has been found outside of Oakland County, and is the first known occurrence of the disease in western Michigan.

Scouting was supplemented by an educational campaign among the school children. A great deal of material was sent in for examination from all sections of the state, especially from the northern counties. Although no blister rust infections were located, the campaign aroused much interest in the protection of white pine.

The first blister rust infection on pine in Michigan was found in a nursery near Pontiac, Oakland County during 1917, a year later than the first reported outbreak of the disease in Wisconsin. Two infected pines were located the following year, one in a nursery near Birmingham and the other in a local ornamental planting. Scouting and inspection work were continued in 1919, but confined principally to white pine plantings and to currants and gooseberries in the vicinity of such plantings. Although the blister rust was not found in the same places where first discovered, another diseased pine purchased from a nursery near Birmingham was located in Oakland County. However, as in previous years all efforts to locate the disease on currants and gooseberries failed. No blister rust was found in the state during the scouting seasons of 1920 and 1921.

Small scattered stands of second growth white pine are frequently encountered in the northern half of the lower peninsula. Oakland County marks about the southern limit of native white pine in the eastern part of the state. In this county where it occurs very sparingly around some of the lakes there are numerous plantations of white pine. However, cultivated black currants are more or less prevalent and a constant source of danger to the pine because of their extreme susceptibility to the blister rust. As this disease is present in Wisconsin and the Province of Ontario, Canada, it was to be expected that the disease would sooner or later invade Michigan. Up to date, however, all infections can be traced to white pine of French origin.

Second growth pine as well as some virgin pine mixed with hardwoods is found in the upper peninsula. Wild and cultivated currants in this region favor the spread of the disease once it becomes established in that part of the state. The pine barrens or plains are somewhat isolated from the sources of infection already known. Wild currants and gooseberries are abundant along the streams and in the swamps, but do not occur on the sand. The state park at Interlochen, as well as many of the state forests are situated on the plains where the localized occurrence of currants and gooseberries will facilitate the control of the rust in these areas.—D. V. BAXTER.

International conference of phytopathologists in Holland, June 25 to 30, 1923. Attention was called to this important meeting in the March number of *Phytopathology* on pages 147-148. More details have been received and the following program is now announced.

Monday, June 25: Presidential address of welcome and lecture on plant diseases as seen from the standpoint of general pathology. Demonstration of experiments, especially on potato diseases. In the afternoon and evening addresses, papers and discussions of foreign and Dutch colleagues.

Tuesday, June 26: Address by Mr. van Poeteren on organization and methods of the Dutch Phytopathological Service. Discussion about international laws and requirements. In the afternoon, demonstrations of some methods of control of pests and diseases as practiced in Holland, e. g. the furrowing-wheel to destroy leather-jackets, control of American gooseberry mildew, of smut and stripe in grain crops, etc. Evening meeting: addresses, papers and discussions.

Wednesday, June 27, and Thursday, June 28: Trips through the country. To prevent congestion of the program, participants are requested to subdivide,—into those interested in agriculture, and those interested in horticulture. On Wednesday morning the first group will travel by rail to Friesland, where farms and selection fields of potatoes are to be visited; next day at Gröningen Agricultural Experiment Station, address of Mr. Hudig on diseases of crops on alkaline and sour soils, and visit to Mr. Hudig's experiments. Visit to the Experimental Farm of the Gröningen Seed Growers' Association or another farm of the same kind.

The second group will visit: first the nurseries of Boskoop and Aalsmeer, situated in a typical Dutch country region below sea level; thereafter visit to the bulb-growing districts near Haarlem. Dr. v. Slogteren will address the company and give a demonstration of modern methods of combating some bulb diseases, e. g. eelworm. This trip will wind up with a visit to the early potato districts of North Holland, where selection is practiced.

It would be possible, if desired, to form a third group for visiting other centers of culture, such as the Westland, the most important district growing vegetables and fruit under glass (grapes and peaches) of Holland (where results obtained by a winter spray with carbolineum against peach and grape scale will show at best advantage); afterwards the orchards in North Holland and the early potato selection fields in the same district might be visited.

Friday, June 29: Meeting of Baarn; address of Prof. Westerdijk on the cultivation of parasitic fungi and demonstration of research work. Afternoon devoted to discussions and inspection of the international collection of fungi cultures. This meeting will be prolonged during the evening for addresses, papers and discussions, and even to Saturday morning, if desired.

Saturday, June 30: In the afternoon the conference will leave for the Hague, for an informal meeting at the Office of the Minister of Agriculture (reception by or on the part of the Minister). Thereafter the committee will be pleased to entertain all participants at dinner in the bathing resort Scheveningen.

The Committee in charge of the Conference is composed of—Dr. H. M. Quanjer, Prof. Johanna Westerdijk, N. van Poeteren, T. A. C. Schoevers, J. Hudig, Dr. E. van Slogteren, G. Kruseman, J. C. Dorst, J. G. Hazeloop, E. H. Krelage, and J. H. van Straaten van Nes.

Opportunity will be given for the reading of papers or for the discussion of important questions on the subject of plant diseases or insect pests, and persons desiring to present papers or to have discussion on any such subject should submit abstracts, in advance of the meeting, to the Secretary, Mr. T. A. C. Schoevers, Villapark 8, Wageningen. Mr. Schoevers should also be notified in the case of each one planning to attend the conference, in order that proper arrangements for transportation and entertainment may be made. It is hoped that as many pathologists as can will endeavor to be in Holland during the latter part of June.—R. J. HASKELL.

Polyporus spraguei Berk., cause of heart rot: *Polyporus spraguei* causes a brown friable rot in the heartwood of the trunk and roots of living *Quercus rubra*, *Q. prinus*, *Q. coccinea*, *Q. velutina*, *Q. marylandica* and *Castanea dentata*. In a mill yard near Salem, Indiana, 16 logs recently cut from living chestnut trees were infected with this fungus. Sporophores developed from the decay in the ends of the logs in about two weeks after the logs were cut. Dr. G. G. Hedgcock, and Mr. G. F. Gravatt both report finding the fungus growing from the heartwood of living oaks. The dark brown friable decay in advanced stages is uniform in the heartwood and is confined to the roots and the lower six to nine feet in the trunk. The fungus fruits abundantly from stumps after trees are cut and may so continue as long as there is any sound heartwood.—JAMES R. WEIR.

Leaf spot of lettuce. Apparently an undescribed *Cercospora* leaf spot of lettuce (*Lactuca sativa*) has been found at the College of Agriculture, Los Baños, P. I. It was first noticed about the middle of March, 1922 and disappeared the last of May when the plants died of old age.

The lesions at first appear as small watery spots at the edge of the leaf. Gradually the spots spread inward and the older diseased tissues turn brownish. The fruiting structures, consisting of conidiophores and conidia, are found on this dead tissue. The conidiophores measure from 63.2 to 130.3 μ long 6.3 to 7.5 μ wide with 5 to 8 septations; the conidia measure 43.4 to 96.3 μ in length, 4.7 to 5.6 μ wide with 5 to 12 septations. The brownish conidiophores are produced in bunches, are simple and erect, and are frequently geniculate as is common in this genus. The conidia are straight or slightly curved, hyaline, tapering from a rather wide base to a point, distinctly linear.

The disease is not fatal to the plant but at times every leaf is spotted and in many cases killed. I have searched through all available literature and I fail to find reference to such a *Cercospora* disease on lettuce. Therefore I suggest the name *Cercospora lactucae* n. sp. to designate the fungus. I am giving this name purely for convenience and I realize its use will be but temporary in that various *Cercospora* diseases have been found to be caused by the same organism on many different hosts.
—COLIN G. WELLES.

Personals: Doctor O. A. Reinking has resigned from the University of the Philippines to take a position as plant pathologist in Honduras, for the United Fruit Company. Doctor Reinking recently received his doctorate at the University of Wisconsin where he has continued researches begun in the Philippines on coconut bud-rot. He obtained leave from the University of the Philippines about a year ago and returned to this country by way of British North Borneo, Java, Italy, Germany, France, and England, visiting important mycological and pathological institutions.

ABSTRACTS OF PAPERS PRESENTED AT THE FOURTH ANNUAL MEETING OF THE CANADIAN DIVISION, AMERICAN PHYTOPATHOLOGICAL SOCIETY, STE. ANNE DE BELLEVUE, PROVINCE OF QUEBEC, DECEMBER 7 TO 8, 1922.

La campagne contre les sauterelles dans Quebec in 1922. G. MAHEUX.

Plant diseases of 1922 in western Quebec. B. T. DICKSON.

Fifty diseases are mentioned among which the following are of interest as being new or of augmented importance during the season. Pink root of onion (*F. mali*) was noted for the first time from Quebec, the wilting leaves being later attacked by *Botrytis* sp. Celery Blight (*S. petroselinii api*) was serious. Powdery mildew of clover (*E. polygoni*) was of such widespread occurrence that clover fields appeared as if powdered with flour. Notwithstanding this, it was difficult to find the perfect stage. Sunflower stem-rot and wilt (*S. libertiana*) was common and rust (*P. helianthi*) was present to the extent of 75 per cent in Sept. *Phyllosticta* leaf spot on McIntosh apple gave 100 per cent infection in one orchard and Bitter rot (*G. rufomaculans*) on Fameuse was found in two orchards. Exceedingly heavy infestation with *E. pruni* causing bladder-plums occurred on wild varieties. *Septoria paeoniae* var. *berolinensis* was pronounced on Peony causing leaf, petal and stem spot.

The natural control of the green apple bug (Lygus communis var. novascotiensis Knight) by a new species of Empusa. A. G. DUSTAN.

The green apple bug is one of the most destructive pests in the apple-growing sections of Nova Scotia. In certain localities the insect has been mysteriously dying out and a study of its natural enemies shows that this is due to a new species of *Empusa*. The life history and morphology of this fungus have been worked out in detail. Studies concerning the economic importance have shown that the fungus lends itself readily to artificial dissemination.

Control of the onion maggot in 1923. W. J. TAWSE.

The Dominion Entomological Service. A. GIBSON.

Disinfection et parcelles. O. CARON.

The pollination of certain vegetable plants by insects. R. C. TREHERNE.

Wood v. fungi (Demonstration slides). R. J. BLAIR AND J. D. HALE.

Address by Dominion Fruit Commissioner. G. E. MCINTOSH.

The past and future of plant pathology. DR. MELVILLE T. COOK.

(Printed in full in Journal of Quebec Society for the Protection of Plants.)

Report of the Plant Disease Survey. F. L. DRAXTON.

Cultural reactions of some dry root-rot organism. T. G. MAJOR.

Seven species of *Fusaria* were grown on seventeen different media at different temperatures and at different H-ion concentrations to determine the effects on growth characteristics. There is a variation in type of growth, colour production, chlamydospore production, and type and size of the macroconidia on the different media. More luxuriant mycelial growth with more intense colouration occurs at 25° C. than at 20° C. but spore size is practically unaffected. A gradation in growth characters was apparent in some species but the reaction of the medium is not the only factor concerned.

The present status of white pine blister rust in Canada. A. W. McCALLUM

Blister rust is now present in the following provinces—Prince Edward Island, Nova Scotia, New Brunswick, Quebec, Ontario and British Columbia. In eastern Canada while the currant stage of the rust is fairly widely distributed the percentage of infected pines is surprisingly small. In British Columbia rust was first found in the autumn of 1921. This year work done in that province has shown that the disease is generally present in the coastal area and that any attempt at eradication or suppression would be quite hopeless. Rust has been present for at least twelve years in this section of the province. Late in the season rust was found on both pines and currants in the interior at Revelstoke and vicinity. The oldest infection found there was about five years of age. If further work does not show that rust is more widely distributed in this area it may then be possible to adopt methods of suppression.

Red branch of Conifers. J. H. FAULL.

Two diseases new to Ontario. J. E. HOWITT.

Two new diseases were found this summer, namely pink root of onions and white rot of grapes.

Pink root is established in the onion growing marshes southeast of Leamington. At least fifty acres of onions were affected by this disease this year. Many fields contained large patches of diseased plants and the growers estimated their loss at not less than 10 per cent of the crop.

White rot of grapes was found in vineyards near Winona and St. Catharines. This disease may cause very serious damage to certain commercial varieties of grapes. The variety which appeared to be most susceptible was Agawam or Rogers No. 15. Unsprayed rows of this variety had over eighty per cent of the bunches affected and unfit for market. White rot is easily confused with black rot. It can be told however, from the black rot by the fact that it apparently always shows on the pedicels first followed by a wilting of the berries which later develops brown lesions, shrivel up and become covered with light brown pycnidia. One year's experiments indicate that white rot even on very susceptible varieties is readily prevented by spraying with Bordeaux mixture as recommended for black rot.

Control of oat smut. DICKSON, B. T., R. SUMMERBY AND J. G. COULSON.

Co-operative experiments (with the Plant Protection Institute) were carried on during 1922 to test the efficacy of copper carbonate dust and other treatments. Banner and Hulless 709 M. C. were used. Formaldehyde spray adversely affected germination in Hulless 709 M. C. Field counts showed 1½ per cent smut in check Banner as against 0 per cent in plots treated with copper carbonate dust and an average of 49 per cent

smut in check Hulless 709 M. C. against 0.3 per cent in plots treated with copper carbonate dust. The latter treatment was almost as efficient as the formaldehyde spray and less injurious to germination.

Abstract of five-years co-operative experiments in the prevention of oat smut. J. E. HOWITT AND R. E. STONE.

Experiments were conducted to show the effectiveness of the dry formaldehyde treatment for the prevention of oat smut. Forty three trials were made and 3,002 bushels of oats treated. The average results of the five-years experiments: Average amount of smut in crop from treated seed .6 per cent. Average amount of smut in crop from untreated seed 3.7 per cent. Average germination of treated and untreated seed was found to be exactly the same, namely 97.5 per cent.

Tests of copper sulphate dip, formalin sprinkle, formalin spray and the so-called dry formaldehyde treatment for oat smut, copper carbonate dust, 2 ounces per bushel and dehydrated copper sulphate and lime, 2 ounces per bushel. Results: copper carbonate dust and copper sulphate dust did not prove satisfactory in the control of oat smut this year on hulled varieties of oats. Copper carbonate dust gave good control of oat smut with the Liberty hulless oats, without any appreciable injury to the germinative capacity of the treated seed. The so-called dry formaldehyde treatment proved to be one hundred per cent efficient this year under plot conditions.

The control of raspberry mosaic. J. F. HOCKEY.

The results of rogueing experiments on the control of mosaic in commercial plantations have proven sufficiently successful to warrant publicity. Plantations which had in 1921, 4.71 per cent, 11.7 per cent and 31.17 per cent were rogued once and in 1922 there was present in these patches, respectively 0.33 per cent, 1.25 per cent and 2.27 per cent. Several of the mosaic plants found this year were new infections which had not shown symptoms at the time of rogueing, others were stray canes from plants which had been rogued. It is evident that by thorough rogueing of one and two year old plantations the disease can be profitably controlled. Rogueing of fruiting plantations over three years is not generally advisable.

Balsam rusts. H. P. BELL AND J. H. FAULL.

Peony diseases. J. G. COULSON.

A study of the diseases of the peony carried on at MacDonald College, P. Q. has shown it to be subject to the following seven diseases.

1. Botrytis blight (*Botrytis paeoniae* Oud.) most troublesome on the buds causing also a small amount of leaf and stem blight.

2. Cladosporium leaf blotch (*Cladosporium paeoniae* Pass.) estimated infection on all varieties 5 per cent.

3. Septoria leaf spot (*Septoria paeoniae* West var. *berolinensis* Allesch.) a very troublesome disease on leaves and stems. The infection for the falls of 1921 and 1922 ranged from 0 to 85 per cent on different varieties. The average for all varieties was estimated at 20 per cent. This is the first time this fungus has been reported in Canada.

4. "Mosaic" diseases—found on a few varieties but not serious.

5. Two leaf spots of unknown cause.

(a) Characterized by small irregular lead white spots with a comparatively broad purple border. Spots confined mostly to upper surfaces of leaves—Estimated infection—2 per cent. Believed to be due to an undetermined *Phyllosticta*.

(b) Definitely limited irregular yellow spots, 2-8 mm. broad with a dark border. Estimated infection 2 per cent. An *Alternaria* is believed to be the cause.

6. Disease of the underground parts.

One plot of peonies was very heavily infected with a typical root rot with a blackening of the rhizome and base of stem. A *Fusarium* was constantly associated.

Root rot and blight of canning peas. R. E. STONE.

During the summer of 1922 our attention was directed to a serious root rot and blight of canning peas. The disease is particularly prominent in Prince Edward county and the farms engaged in growing canning peas for seed. In many cases only spots in the fields are affected but on some farms whole fields show the presence of the disease and did not produce as much seed as was sown.

Enough experimental work was carried on to show that seed treatment and the use of fertilizers in commercial quantities would be of no benefit.

One of the fungi isolated from the diseased plants was *Fusarium* sp., but it is probable that other fungi may be associated with the trouble.

At present the plans suggested for combatting the spread of the disease include; 1. A long period of rotation, 2. Thorough drainage, 3. Use of resistant strains of peas.

Distribution of Ribes and Cronartium ribicola in Ontario. G. H. DUFF.

Bacterial soft rot of Iris. J. K. RICHARDSON.

The disease is a typical soft rot, destroying the rhizome as well as the foliage. Thirty-six rot-producing organisms were isolated from various sources and inoculations carried out, which resulted in obtaining typically diseased plants from the inoculations with two of the isolated organisms. These two reacted in a similar manner to, and appear to be, forms of *Bacillus carotovorus* Jones.

Blue stem of the black raspberry. J. F. HOCKEY.

The blue stem disease of black raspberry as originally described by Lawrence (Wash. Agr. Experiment Station Bulletin 108, 1912) has been found in commercial plantations scattered through the Niagara district. A fungus growth of the *Verticillium* type and corresponding to the description of *Acrostalagmus caulophagus*, Lawrence, was readily isolated in culture and is now under observation and study. It appears from Carpenter's article in the *Journal of Agricultural Research* 12: 529-546, as though this organism differs very little from and probably is identical with *Verticillium albo-atrum*, Reinke and Berthold.

Plant pathology in public schools. W. A. McCUBBIN.

Dusting with copper carbonate and other substances for smut control. W. P. FRASER AND P. M. SIMMONDS.

Experiments were carried out in co-operation with the Dominion Experimental Farms at Indian Head, Rosthern and Scott in Saskatchewan and at Lacombe in Alberta. Copper carbonate dust, copper sulphate and lime and a number of other substances were used and compared with the wet formaldehyde treatment. In all cases the formaldehyde solution gave perfect control. Copper carbonate dust was fairly effective in the wheat experiments, reducing the bunt present to about $\frac{1}{2}$ per cent. With Banner oats the smut was reduced by copper carbonate to about one per cent. In the experiments with Liberty oats (hulless) which are seriously injured by formaldehyde treatment, copper carbonate dust reduced to smut to a small percentage except where the

check plots showed a very high percentage of smut. Copper sulphate and lime and the other substances used for dusting were not satisfactory.

Treatment of wounds in tree surgery. R. E. COSSETTE.

The bronze birch borer. C. B. HUTCHINGS.

Preservative treatment of farm timbers. J. H. CODERRE.

A contribution to the knowledge of the tree destroying fungi of the Vancouver forestry district.
N. L. CUTLER.

A study was made of tree destroying fungi in the region of Vancouver. The following 24 species were found to be important:

Fusicoporia ferruginosa (Schrad.) Murr.

Fomitiporia tsugina Murr.

Poria sp., *P. subacida* Pers.

Fomitiporella betulina Murr.

Coriolus versicolor (L.) Quel., *C. delectans* Murr., *C. balsameus* (Peck) Murr.,
C. abietinus (Dicks.) Quel.

Piptoporus suberosus (L.) Murr.

Polyporus fissus Berk.

Laetiporus speciosus (Batt.) Murr.

Phaeolus sistotremonides (Alb. & Schw.) Murr.

Fomes roseus (Alb. & Schw.) Cooke, *F. unguatus* (Schaeff.) Sacc., *F. laricis* (Jacq.)
Murr.

Pyropolyporus robiniae Murr.

Porodaedalea pini (Thore) Murr.

Elfungia megaloma (Lev.) Murr.

Ganoderma oregonense Murr., *G. sessile* Murr.

Daedalea confragosa (Bolt.) Pers.

Gloeophyllum trabeum (Pers.) Murr., *G. abietinellum* Murr.

Twenty four species of fungi were found associated with timber rots in this district. Of these, six belong to the tribe Poriae, eight to the tribe Polyporae, eight to the tribe Fomitae, three to the tribe Daedaleae, one to the tribe Ascomycetes.

The species were distributed on the various hosts as follows:

On *Pseudotsuga taxifolia* 7 species

Tsuga heterophylla 12 "

Tsuga mertensiana 1 "

Picea sitchensis 2 "

Acer sp. 1 "

Acer macrophyllum 1 "

Alnus rubra 2 "

Rhamnus purshiana 1 "

Host unknown 3 "

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THE BIOLOGICAL BASIS OF FOREIGN PLANT QUARANTINES

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Plant diseases and insect pests are often limiting factors in crop production and their prevention or control is one of the major problems of agriculture.

In all developed countries the introduction and spread of such pests in the past has caused such heavy losses, even to the point of restriction or abandonment of important crop industries, and now imposes such a heavy annual tax for sprays, seed treatments, cultural control and the substitution of resistant varieties of cultivated plants that the people have invoked governmental aid to apply official regulatory measures which will protect them from additional burdens of this character in the future.

The efforts that are being made along this line may be grouped under four heads, according to the prevalence status of the pests involved:

1. *State Inspection and Certification of Plants and Plant Products.* All progressive countries maintain inspection services for the general control of insects and plant diseases, including those already common, widespread or endemic, through supervision of commerce in nursery stock, seeds and plant products.

2. *Eradication.* To wipe out newly introduced parasites presenting an unusual menace to our crop industries. Two outstanding illustrations are the fight waged against citrus canker in Florida and that against the pink bollworm of cotton in Louisiana and Texas. The eradication method has also been applied successfully to outlying "long jump" infestations of gypsy moth and other insects. As a rule, it is extraordinarily difficult to eradicate an insect or disease that has gained a firm foothold in a region favorable for its maintenance.

3. *Domestic Quarantines.* Restriction of new introductions by local or domestic quarantines drawn around an area where a new pest from a foreign country has become established. Examples are the quarantine against the gypsy and browntail moth in New England, the Japanese beetle in New Jersey and Pennsylvania, and potato wart in three states.

¹ Read at the joint meeting of the American Phytopathological Society and American Association of Economic Entomologists, Boston, Mass., December 30, 1922.

In the same category may be placed the efforts made by various states to keep out pests established in other, often distant states. Arizona regulates against cotton pests from the East, alfalfa weevil from the North, citrus pests from the South, and grape phylloxera from the West. In such cases a barrier has been erected around the pest-free area rather than around the infested area.

These local or domestic quarantines are temporary expedients, whereby the spread of a pest is slowed up or held back at heavy cost and much inconvenience, but domestic dispersal is almost unrestrainable and, sooner or later, the point is reached where the newly established pest acquires the status of other widely distributed parasites, to which we have to adjust ourselves forever.

4. *Foreign Quarantines.* These seek to exclude the plant pests of other countries on the principle that frontier boundaries are the most effective fighting lines. It is primarily with this problem that we are to deal in this paper.

We stand in danger of subjecting ourselves to ineffective and hampering regulations without adequate gain unless the quarantine procedure is based upon a firm scientific foundation of sound biological principles derived from properly coordinated facts relating to the nature of the parasites to be dealt with, their country of origin and geographic distribution, their host relations, and the native home of the host, prevalence, climatic relations, manner of spread, and other factors. More studies of this character need to be made, but enough is known to enable some general principles to be established.

FOREIGN QUARANTINE PROBLEMS DISTINCT

It is our purpose to emphasize that the application of biological principles to plant quarantines must lead to a primary distinction between the problem of control of spread within a given area, which has been termed "continuous spread," and the long distance transfer that has been termed "discontinuous spread" by Butler¹ in his excellent monograph on this subject.

It was pointed out in 1914² that we have to deal with an *intercontinental* problem and that to safeguard the exchange of plants and their products between countries situated in different continents more stringent precautions might become necessary.

¹Butler, E. J. The dissemination of parasitic fungi and International Legislation. *Memoirs of the Department of Agriculture in India*, 9: 1-73, 1917.

²Orton, W. A. The biological basis of international phytopathology. *Phytopathology*, 3: 327, 333, Feb. 1914.

That in establishing plant quarantine policies the problems of restricting parasites from foreign lands must be grouped separately from the problems of preventing or delaying dispersal of pests already locally established may appear self-evident to some, but that this important point is not yet properly acknowledged is shown by the report of the last International Phytopathological Convention held in Rome in 1914, which makes no distinction between contiguous and non-contiguous countries, with respect to the methods to be adopted to prevent the spread of plant parasites. The representations made by European delegations to the Federal Horticultural Board during the past year have likewise ignored this basic principle, and have included proposals for intercontinental exchange of plants that we must regard as extremely unscientific and dangerous.

THE BASAL PRINCIPLE OF BIOLOGICAL EQUILIBRIUM

The plant parasites of one continent or natural plant zone are likely to be more virulent and destructive when introduced into another continent or plant zone than they are at home, where they are in equilibrium with their hosts and associates. This principle of biological equilibrium in Nature is best understood by considering from the viewpoint of evolution the results of the complex interactions of organisms, the struggle for existence, the influence of animals on plants, of plants on animals, and the disturbing effect of man, who seeks to exterminate some animals and plants and increases the number of other organic elements in the equation, even bringing in foreign organisms to participate in the mixup.

In the course of evolution many marvelous adaptations to environment arise and many complex interrelations develop. Organisms are sheltered by some of their associates and preyed upon by others. They become dependent upon certain kinds of food, and increase or diminish in numbers with the abundance of this food.

The type of relations that concerns us is *parasitism*, wherein one organism lives upon and at the expense of another. A classification of parasites on the basis of their adaptation to their hosts reveals the course of evolution, which has progressed from saprophytism, through wound parasites and weakling parasites, toward a stage of closer adaptation between host and parasite till symbiosis is reached, a relation where the parasite does little or no injury or even stimulates the host to larger growth till the reproduction and dissemination of the parasite is assured.

There is a tendency for the fungus or bacterium to reduce its toxin formation, and on the part of the host to develop a degree of immunity, so that a balance is established between the two, with a lessened injury

to the host and a more highly perfected mechanism of dispersal for the parasite. With insects, the balance is established more often through the influence of parasites, predatory insects, and birds.

This equilibrium is dynamic, not static, ever changing, but tending to preserve the status quo, and in the establishment of equilibrium the time element is important. While the restoration is slowly taking place in a new country much economic damage often occurs.

So it comes that the natural parasites of a region are not highly destructive to the plants of that region, as a general rule, but when one of these parasites is transferred to a different plant zone, it may come into contact with a new set of organisms and find new hosts that are not resistant.

In considering the problems that arise when a new plant disease or insect is discovered to have been introduced, or in estimating the risk presented by a foreign parasite, it is well to begin by ascertaining:

1. The country where the parasite is endemic.
2. Its native or original host.
3. The geographic distribution of this host.
4. Its relationship to other economic plants in our country.
5. If the host is cultivated here, to what extent and for how long it has been subjected to plant breeding that may have resulted in the production of distinctively American strains whose resistance to the parasite is unknown.

Various types of disturbance of biological equilibrium by the transfer of parasites from one plant zone to another occur and may be illustrated by notable examples.

I. A parasite introduced from one continental zone to another finds susceptible hosts. A conspicuous instance where a foreign fungus has found an American host more susceptible than its original host is the chestnut bark disease, *Endothia parasitica*, which occurs in Asia on *Castanea mollissima* as a relatively minor trouble, but introduced into the United States by accident, has attacked our *Castanea dentata* most virulently and will practically destroy all the chestnut forests of the country before it stops. In America this Asiatic parasite has found the European chestnut, *Castanea vesca*, as easy prey as the American and if introduced into Europe it may yet repeat there the devastation we have witnessed here. Europe has already suffered heavily from introductions of this type. The wine grape, *Vitis vinifera*, has been scourged in succession by the phylloxera, the downy mildew, *Plasmopara viticola*, and the black rot, *Guignardia bidwellii*, to all of which American species of *Vitis* are resistant.

European gooseberries are found to be susceptible to the American mildew, which is becoming widespread in Europe. We have long known that the European varieties were relative failures in the United States on account of this mildew, to which American species are resistant.

The white pine blister rust, *Cronartium ribicola*, a native of Europe on *Pinus cembra* and other species is very destructive to *Pinus strobus* in the United States, and another American species, *Pinus monticola*, is several times as susceptible as *P. strobus*. This had been forecasted prior to the actual introduction from the injury done to American white pines when planted in Europe.

The hollyhock, a native of China, met the rust, a South American fungus, in Europe and its culture was nearly stopped. The disease later spread to North America and repeated its destruction here.

In this general category belong also numerous cases of insect transfers to other zones where, relieved of the enemies that have held them in check at home, the invaders have multiplied with great rapidity. The cottony cushion scale, *Icerya purchasi*, for example, came to California from the Orient and was not controlled till its enemy, the Australian lady bird *Vedalia cardinalis*, was brought over and a new biological equilibrium established. Similarly the Japanese beetle, *Popilia japonica*, the European corn borer, *Pyrausta nubilalis*, the gipsy moth, *Porthetria dispar*, the brown tail moth, *Euproctis chrysorrhoea*, and the alfalfa weevil, *Hypera postica* came to this country without their natural enemies. In combating them efforts have frequently been made to discover and to introduce artificially their enemies.

II. A plant, carried from one continental zone to another, is attacked by new parasites. The culture of maize in Java and the Philippines is well nigh impossible because of the corn mildews, *Sclerospora spontanea*, *S. maydis*, *S. sacchari*, and *S. Philippinensis*, which are oriental species, the latter being native on *Saccharum spontaneum* and *Miscanthus japonicus*.

When the common potato in its spread from South America reached the Rocky Mountains of the north, it was attacked by the Colorado beetle, *Doryphora decemlineata*, which found the newcomer more palatable than the Solanums on which it had been accustomed to feed.

Of the same type is the situation we are now facing in Arizona, where a native weevil, *Anthonomus grandis* var. *Thurberiae*, related to the cotton boll weevil, has already shown an inclination to move over to the cultivated cotton, a relative of its wild host, *Thurberia thespesoides*. Since the *Thurberia* weevil is acclimated to the dry southwestern climate as the Mexican weevil is not, it would extend the zone of weevil injury as far as cotton is grown.

As already mentioned, the European gooseberries brought to American were attacked by the American mildew and the attempts to establish the culture of European grapes in eastern North America were always failures on account of phylloxera, black rot, and mildew.

III. A cultivated plant, transferred from one continent to another without its parasites, is later followed by them. In the interval, new varieties have arisen which do not possess the high resistance the species originally had. Our American agriculture is based so largely on introduced crops that we have had numerous cases of this type in the past and there is ground for grave apprehension that still others may be brought in. The asparagus rust affords a significant illustration of this phase of this biological principle. Asparagus and its rust are both native Europeans and the disease is not highly injurious on that continent, but the crop came to the United States without the disease and from its earliest culture until about 1896 was free from rust. During this period American varieties were bred and formed the basis of our industry when the rust finally invaded the United States. It soon transpired that the Americans strains of asparagus which had been developed in the absence of the rust and never before had been in contact with the disease were very susceptible, and they were soon wiped out. European strains were imported and resistant types selected from them. These have now replaced the old susceptibles kinds, and an interesting thing has happened. Asparagus rust has dwindled and in places almost disappeared; in other words, we have restored the natural equilibrium such as exists in Europe.

IV. Domestic parasites are sometimes limited in their natural geographic distribution by climatic factors. The black rot of grape, which requires high temperatures for infection, is most at home in the central eastern states. A New England grape from *Vitis labrusca*, the Concord, is more susceptible in that region than are varieties derived from *Vitis rotundifolia*. Pecan scab, *Fusicladium effusum*, is most destructive to varieties from the southwest, Texas for instance, planted in the more humid region of the Atlantic Seaboard.

The vast areas planted to one crop in the United States make for a disturbed equilibrium and afford favorable conditions for the rapid spread of parasites.

The transfer of plant parasites from continent to continent was extremely slow in the early days when the means of communication were scanty and the voyages long, but notable instances have multiplied in proportion as the volume of traffic has increased. World trade is certain to be in a still larger scale in the future, with proportional risk of spreading pests unless scientific control measures are rigidly enforced.

NATURAL BARRIERS AGAINST PLANT PESTS

What are the plant zones within which organic life is in a state of equilibrium? Clearly they are in the main *continental*. The extent to which mountains and deserts restrict the spread of parasites has never been adequately studied but such cases are known. Here is a field to be highly commended to investigators.

It will be clear that the plant relations of two contiguous countries without a natural barrier are different from those of two countries separated by an ocean barrier. In the first instance the endemic parasites are the same and respect no political boundaries. The plants and parasites of the United States have reached their ultimate distribution except as new introductions are still spreading. For the countries of Europe the Rome Convention may be useful. A similar plan of certification is in effect between our states but it will not cover the relation between North America and Europe, and still less between North America and Asia.

Since plant parasites are in dynamic equilibrium with their natural hosts, with a tendency toward reduced injury, it follows that it is now impossible and that it never will be possible to foretell from the behavior of the disease or insect in its native home what it may do when transferred to another continent. Experience has shown that in many cases we may expect it to go over to a near related host and attack this plant with great virulence.

It is important to study in Europe and Asia the diseases of American plants that have been introduced there. For example, we knew in advance that the European blister rust was especially destructive to American white pine, because that tree had been much planted in Europe and had failed there because of the rust.

The effect of the canker due to *Phomopsis pseudotsugae* recently reported from Scotland¹ upon the American species of *Pseudotsuga*, *Larix*, *Tsuga*, and *Abies*, now growing there deserves careful study in the light of the importance of our western Douglas fir forests.

Most of our cultivated plants are native to other continents, but crop plants are subject to rapid change by breeding and the resistant character previously attained abroad may be dropped or diminished unawares. Since our present day varieties are of American origin, bred for adaptation to our climate and soil, it is to be expected that as additional parasites accidentally become established they will be more destructive to our American bred varieties than they are to the parent

¹ Wilson, Malcolm. A new species of *Phomopsis* parasitic on the Douglas fir. Trans. Bot. Soc. Edinburgh, 28: 48-50. 1920.

species in the original habitat. The need for strict exclusion is therefore so much the greater.

CROP INTRODUCTION

To promote the agriculture and horticulture of a new country, it is desirable to introduce the crop plants of other lands. It is possible, and highly desirable, to accomplish this without bringing along their parasites. For example, we have the Australian eucalyptus on the Pacific Coast with very few pests, whereas in its native country it has a good many parasites. This is probably due to the fact that our introductions have been by seed rather than by plants. Unfortunately, this freedom from pests can be said of few of our great staple crops. Many diseases of wheat, potatoes, apples, peaches, etc. trace back to the original habitat of these crops. What losses beyond the possibility of estimation might have been saved if national barriers against plant pests had been erected at the start. However, by no means all of the crop pests of other lands have been imported, and we have still many new plants to bring in. It is to be hoped their enemies may be left behind.

At this point we may expect to hear from the man who proposes that it would be better to let all the foreign parasites in and strike a balance with them, developing control method once for all.

Unthinkable! Count the cost of a few more scourges like the chestnut bark disease. Could our corn growers combat the Philippine downy mildew? In his description of the ravages of this disease in the Philippines, Weston describes fields where practically every plant was destroyed and he believes that climatic conditions in our corn belt would favor the mildew. It is difficult enough to establish the culture of a new crop in the absence of its serious pests. It would become impossible if all the enemies were brought in.

Moreover, a biological equilibrium would never get established, for the succession of possible pests from all the ends of the earth is endless. There would always be more to come. We would not learn to cope with one enemy before another would be upon us.

Control of pests is not an attempt to overcome nature's laws. The movement of cultivated plants into new regions is a reassembling of environmental forces (geological, climatic, biological, anthropological) to permit production of material useful to man. The economic plant product is the resultant of these forces. The importation of plants without the importation of pests is merely a reassembling of these forces in a different and just as natural a manner as the importation without regard to the transfer of pests.

WHAT ARE THE FACTORS GOVERNING THE SPREAD OF PLANT PARASITES?

Theorists might stress wind carriage. Volcanic dust has been borne thousands of miles through the air. Why not fungus spores?

But no case of long distance transfer of plant disease by wind has been demonstrated, and the facts of geographic distribution show that diseases frequently have failed to spread even short distances until carried by man. There is a place in the District of Columbia where for twenty years apples and red cedars have been growing in close proximity without a trace of cedar rust, though ten miles away this fungus is a scourge to both hosts. Butler, after an extensive review of the evidence, concludes, that "infection by spores carried through the air from remote centers is not a contingency which needs to be seriously taken into account."

Ballast brought to our shores in the holds of vessels from foreign ports is a bugaboo to the theorists, but no case of disease importation has been traced definitely to it. The practice should be regulated and ballast of live or agricultural soil prohibited. Earth balls on the roots of imported plants stand in a very different relation and are much more dangerous, as they are the resort of many insects and may contain harmful fungi or nematodes.

Spores have been found on birds, and birds fly long distances. Clearly they might spread infection, but the facts of distribution already referred to and studied, of the spread of great plant epidemics, indicate that spread is mainly by continuous dispersal, or in long jumps through human agencies.

PLANT DISEASES SPREAD BY MAN

The most frequent agent in plant pest dissemination is man, and the outstanding and altogether most important medium of transfer is living plants or parts of plants for propagation. Trees, roots, bulbs, and seeds, taken in a living condition from their place of origin, with parasites attached, are carried to a new location, set out, and brought into growth. The careful moist packing and cultural precautions taken to preserve their vitality likewise insure the perpetuation of the parasite.

Insufficient consideration has been given to dispersal on seed through external or internal infection, which has carried many plant parasites over the world. War needs brought a shipload of castor beans from India to Florida, and with it a *Botrytis* blight that made the culture of this crop impossible. Investigation proved that the parasite was seed-borne.

Practically all of the pest transfers of which we have accurate record have taken place in this way. In the nursery a large number of related

species of plants are brought together in such a way as to favor continuous spread. If these plants become infected the discontinuous spread of the disease may occur when the nursery stock is shipped to distant parts of the world. Thus in the majority of cases the nursery is the principal center of distribution.

We must lay it down as an unchangeable law of nature that *inter-continental trade in plant propagating material* is fundamentally dangerous, and to be held within the narrowest limits compatible with economic need.

In human diseases the same principles apply. The living carrier is a greater risk than air-borne infection. The practices of the most progressive public health authorities show an increasing recognition of this principle.

INSPECTION INADEQUATE

Experience has shown that by an inspection of plants in nurseries, or better in the packing house, a worth while safe-guard is provided for shipments to contiguous states in the same biological zones, since the number of parasites is greatly lessened, but the most rigid inspection can not detect all the insects, as shown by the frequent findings of brown-tail moth nests in certified nursery stock upon its arrival in America from North Europe. How much less likely are the inspectors to discover the presence of fungous and bacterial diseases, due to organisms of minute size and often embedded in the tissue of the host plant, as in the case of white pine blister rust, where an internal infection of seedlings shipped to America from Europe has been detected two or three years later.

There are three biological factors which operate to make inspection inadequate for intercontinental shipments.

1. Human powers of observation are not keen enough to detect 100 per cent of visible infection.

2. Some parasites are internal, without outward manifestation.

3. Organisms common and of minor consequence in one biological zone may become destructive parasites in another biological zone.

The tendency of inspection officials has been to overlook "common species of long standing dispersion," to quote the language of the Rome Convention, which in addition omits from consideration "enemies of plants whose ordinary supports do not exist in the importing countries," and those which do not present:

1. An epidemic character.

2. A destructive or at least very harmful action on cultivated plants.

3. An easy propagation by living plants or parts of living plants.

These considerations are logical only when applied to contiguous countries. To other continents the only effective protection is the nearest approach to absolute exclusion of living plants that economic needs permit. Importations for propagation of new or rare varieties should be under governmental supervision, and if the origin is in unsurveyed countries they should be grown for a period in a quarantine or detention station, and in any case should be given the most effective treatment possible.

TREATMENT METHODS INADEQUATE

Fumigation of plants is a safeguard, but not a complete protection. It has not reached scale insects in tightly wrapped leaves or buds, as in the date palm, nor egg stages of the cane leafhopper, nor insects in moist earth or in packing material around plants. Fumigation has also failed completely to kill the larvae of the twig borer, *Laspeyresia molesta*, in peach twigs and those of the corn borer, *Pyrausta nubilalis*, hidden in the stalks of bales of broom corn.

No method has been devised to disinfect seeds or plants that can be depended upon to reach all fungi and bacteria present, though such treatments are of value in particular cases.

WHAT THEN OF PLANT PRODUCTS FOR FOOD OR MANUFACTURE?

This is an important question, because such commodities form a large part of our trade, both export, import, and domestic, and extensive restriction might prove more harmful than helpful.

Here again, proof is lacking, except in a few instances, that plant pests are actually disseminated, and these cases are mostly those where the commercial product is a seed, tuber, or part capable of propagation. Cotton seeds are dangerous carriers of pink bollworm. Potatoes introduce disease to the extent that table stock is used for planting. Tuber parasites are transported with particular ease. Apple fruit has not spread San Jose scale. This scale has not become established in northern Europe, although often present on fruit imported from Australia. Pear fruit does not expose the importing country to a risk of pear blight. Fruit flies, however, are carried in commercial fruit and may escape therefrom and are therefore subject to discontinuous spread. Each case is a problem for research and separate consideration.

It highly behooves those interested in building up an intercontinental trade in plant products to take measures to provide a safe foundation for such enterprises, to insure that the importation of a fruit or vegetable or other plant product will not later become a means of exposing North American crops to injury from a new disease or insect.

This foresight can be exercised through scientific research in the country of production, in tropical America, for example, to keep out pests or to keep them down, or to develop methods of culture and packing that will make it impossible for pests to be spread.

SUMMARY

Plant quarantine policies and methods must be founded on nature's laws governing the dispersal of parasites.

The problems of discontinuous spread between countries separated by ocean or other natural barriers are therefore different from those of continuous spread in adjacent countries.

The pests of other continents are fundamentally the most dangerous. Seldom can they be known in advance or completely eliminated by inspection. Consequently intercontinental trade in living plants will always be attended by such grave risks that it should be held within the narrowest limits compatible with economic needs.

FEDERAL HORTICULTURAL BOARD,
WASHINGTON, D. C.

BACTERIAL SPOT OF TOMATO AND PEPPER¹

MAX W. GARDNER AND JAMES B. KENDRICK²

WITH PLATE XIV AND ONE FIGURE IN THE TEXT

At the time of our previous reports (3, 4) on bacterial spot of tomato, the identity of the causal organism of pepper bacterial spot was only partially established and the final report of Miss Doidge on tomato canker in South Africa was not accessible. It is our present purpose to clear up the uncertainty relative to the identity and taxonomy of the causal organism and to record the results of our further study of the disease in Indiana.

HISTORY OF THE DISEASE

The earlier literature was summarized in our previous report (3) in which we described the tomato organism, recorded successful reciprocal cross inoculations with the causal bacteria of the tomato and pepper diseases, and indicated that the organisms were probably identical. A few months before our account was published, Miss Doidge at Pretoria, Transvaal, published a preliminary (1) and a detailed (2) description of a bacterial tomato canker occurring in that region which has proved to be identical with the disease studied in Indiana.

The bacterial spot of pepper described by Sherbakoff (7) in Florida was observed at Sanford, Florida, by I. C. Jagger in 1920 and 1921 and was reported from Tennessee by Sherbakoff in 1921. Takimoto (8) has reported a seed borne bacterial spot disease of peppers in Korea but he isolated a white bacillus entirely different from the yellow monoflagellate form which causes the disease under consideration. Recently the disease has been studied and described in detail by Higgins (6) in Georgia. It occurred near Indianapolis in the fall of 1922 in a crop of peppers grown under overhead irrigation.

In brief it may be said that the disease as it occurs on both hosts is favored by wet weather, that leaf infection occurs through the stomata, that the bacteria are intercellular and the host cells are stimulated toward hyperplasia, particularly in fruit lesions, and that the bacteria are carried on the seed and consequently the disease may be controlled by seed disinfection.

¹ Contribution from the Botanical Department, Purdue University Agricultural Experiment Station, LaFayette, Ind.

² The writers wish to acknowledge their indebtedness to Prof. H. S. Jackson for helpful suggestions.

IDENTITY OF THE CAUSAL ORGANISM

Miss Doidge described the causal organism as *Bacterium vesicatorium* n. sp. in her final publication (2) which appeared in February, 1921. We did not have access to this before the publication of our description of the organism as *Bacterium exitiosum* n. sp. in April, 1921 (3). Higgins (6) did not decide upon the nomenclature of the pepper organism. A comparison of the three descriptions shows that all agree that the organism is a monoflagellate, aerobic rod which forms yellow colonies, liquefies gelatin, and produces no gas from carbohydrates. In fact there are only a few really important differences, such as the toleration of acidity and the production of acid from certain carbohydrates.

In order to establish the identity of the organism, a culture (B) of *Bacterium vesicatorium* obtained from Miss Doidge in July, 1921, and a culture (C) of the pepper bacterial spot organism isolated from leaf lesions at Sanford, Florida, on April 21, 1921, by Dr. I. C. Jagger were compared in a series of tests with a culture (A) of our tomato organism. The three strains were found to be essentially similar in appearance and behavior. Cultures on beef agar, potato agar, potato cylinders, and gelatin were similar. In milk with litmus or methylene blue the color was destroyed and the milk was digested without coagulation. All liquefied plain blood serum and Loeffler's blood serum, although the pepper strain (C) failed to produce the brown discoloration noted in the A and B cultures.

All tolerated 3 per cent sodium chloride in neutral beef broth and were inhibited by 4 per cent. The tolerance of acidity was determined by growth in beef broth cultures in which the amount of acid (Fuller's scale) was determined by titration with NaOH with phenolphthalein as an indicator and the hydrogen ion concentration was determined by titration with the sulphone phthalein indicators brom phenol blue, methyl red, brom cresol purple, brom thymol blue, and phenol red.

In terms of Fuller's scale, all strains tolerated HCl up to + 12 and culture C (pepper) endured + 15. All tolerated HCl up to pH 5.0 and were inhibited by a pH of 4.8. All tolerated malic acid up to pH 5.0 but were inhibited by a pH of 4.5. All were inhibited by a pH of 4.8 obtained by adding acetic or citric acid. The three strains grew in broth rendered as alkaline as -25 with NaOH and grew in broth to which NH₄OH was added until a pH of 8.8 was shown with cresol red.

The principal differences in the published descriptions related to the production of acid from carbon compounds. The three strains were grown in test tubes containing 2 per cent solutions of dextrose, saccharose, maltose, lactose, glycerin, and mannit made up in a 2 per cent peptone solution and adjusted to a pH of about 7.0. One series contained the

indicator brom cresol purple, one, brom thymol blue, and the third, phenol red. The three strains behaved similarly and in no case was there any evidence of acid production. There was a marked increase of alkalinity in the mannit and glycerin cultures while the others remained neutral or became slightly more alkaline.

On litmus beef extract agar slants containing 2 per cent dextrose, saccharose, or lactose, all cultures were similar and no acid production was indicated. In cultures on beef extract agar slants containing the three carbohydrates and the hydrogen ion indicators, there was no evidence of acid production and increased alkalinity was indicated by the red color of the phenol red series, especially in the lactose cultures.

Both Miss Doidge and Higgins found ammonia produced by the organism and to this, Higgins ascribes the failure to detect acid production in the standard media. By reducing the protein content and increasing the sugar content of the media he was able to demonstrate acid production from dextrose, saccharose, and lactose.

However, under the conditions of our tests the three strains were practically identical. Furthermore all proved infectious to tomato and with the tomato strain (A) successful inoculation of pepper leaves and fruit was obtained. Therefore it seems safe to conclude that the causal organism of tomato bacterial spot described in Indiana, of tomato canker described by Miss Doidge, and of the bacterial spot of pepper are identical and by priority rules should be known as *Bacterium vesicatorium* Doidge.

TEMPERATURE RELATIONS

Miss Doidge (2) found that the organism grew throughout a wide range of temperatures with an optimum near 30° C. and the writers (3) found likewise except that the optimum lay between 25° and 30° C.

A loopful of a suspension of the tomato organism (A) was placed in the center of each of a series of 16 poured plates of plain potato agar and after three days incubation at room temperature, the colonies were measured. Two plates were then placed in damp chambers in each of a series of eight constant temperature incubators providing a range of temperatures from 2° to 35° C. At the end of 15 days the long and short diameters of the colonies were measured and the mean increase in diameter at 2° was 5.5 mm., at 9°, 8.5 mm., at 16°, 17 mm., at 20°, 18.5 mm., at 23°, 26 mm., at 27°, 31.5 mm., at 30°, 12.5 mm., and at 35°, 10 mm. These results show that the organism grows in a wide range of temperatures with an optimum at 27° C. as is shown graphically in figure 1.

The thermal deathpoint of the pepper organism (C) was found to be 49° C., very near to that previously reported for the tomato organism (49°-50° C.).

EFFECT OF FREEZING

In order to determine the effect of freezing, water suspensions of the tomato organism (A) were made up in five small flasks and frozen by embedding in crushed ice and salt in a container which was stored in a refrigerator. The temperature of the ice-salt mixture was -10° to -12° C. The number of bacteria per cubic centimeter in each flask was estimated by the poured plate method before freezing and at different intervals certain of the flasks were thawed and plate counts were made. Flask 1 was refrozen once, flask 4, twice, and flask 5, four times. While the counts based on the plates are considered only as approximations, the results are presented in table 1. The percentage of viable organisms in terms of the original suspension is included.

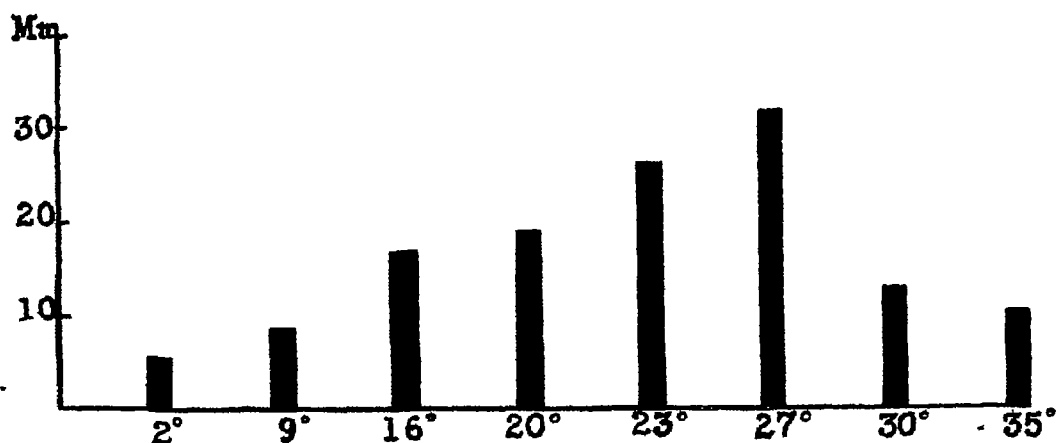


FIG. 1. Effect of temperature on increase in colony diameter of *Bacterium vesicatorium*.

These results show that the mortality increases with the duration of the freezing and with the alternate freezing and thawing. Two to 23 hours of freezing killed 25 to 37 per cent of the organisms, 40 hours, 88 per cent, and 65 hours, 93 per cent. Refreezing 12 hours killed 90 per cent of the organisms remaining alive in flask 1 after the first two hours of freezing, and refreezing six hours killed 56 per cent of the organisms alive in flask 4 after the first 40 hours of freezing. Freezing for eighteen days (flask 5) during which thawing and refreezing occurred four times was sufficient to kill all of the organisms.

It is necessary to admit however that the organism is rather resistant to freezing in water. This is further substantiated by the fact, discussed later, that the organism survived a winter in the field.

HOST RANGE

In our previous report (3) this organism was reported parasitic upon tomato, pepper, and potato. Miss Doidge (2) was unable to infect

TABLE 1—*Effect of freezing in water suspension.*

Time frozen	Flask 1		Flask 2		Flask 3		Flask 4		Flask 5	
	Bacteria per cc.	Per cent	Bacteria per cc.	Per cent	Bacteria per cc.	Per cent	Bacteria per cc.	Per cent	Bacteria per cc.	Per cent
0	2,164,000	100	4,869,000	100	2,770,000	100	2,126,000	100	1,609,000	100
2 hrs.	1,617,000	75								
14 "	165,000	8	2,959,000	61						
23 "					1,742,000	63				
40 "							249,000	12		
46 "							108,000	5		
65 "							28,000	1	105,000	6.5
87 "									78,000	5
8 days									1,800	0.1
16 "									300	0.02
18 "									0	0

lemon seedlings, cotton seedlings, young walnut trees, cabbage, bean, and tobacco seedlings, sweet peas, and *Solanum incanum*. She obtained infection of *Solanum nigrum*, *Physalis minima*, and *Datura stramonium*, var. *tatula*. Higgins (6) reports infection in Pimento, Tabasco, Chili, and bell peppers.

Atomizer inoculation under greenhouse conditions with the tomato organism (A) has yielded only negative results with turnip, cabbage, eggplant, tobacco, *Datura stramonium* L., *Solanum carolinense* L., *S. integrifolium* Poir., and *S. opacum* A. Br. and Bouché. Infection occurred on *Lycopersicum pimpinellifolium* Dunal (currant tomato), *Lycium halimifolium* Mill. (matrimony vine), *Lycium chinense* Mill., *Hyoscyamus niger* L. (henbane), *Hyoscyamus aureus* L., *Nicotiana rustica* L. (wild tobacco), *Solanum dulcamara* L. (bittersweet), and under field conditions on *Solanum rostratum* Dunal (buffalo bur).

Abundant infection of tomatoes with the pepper organism (C) was obtained in the field as was also abundant infection of peppers, including fruit lesions, with the tomato strain (A). In the greenhouse successful inoculation of *Hyoscyamus niger*, *Hyoscyamus aureus*, and *Nicotiana rustica* was obtained with the pepper strain (C).

With the tomato strain (A) atomizer field inoculations in August, 1921, resulted in infection on 32 seedsmen's tomato varieties not included in our previous list of 41 susceptible varieties and fruit infection occurred in all but four. The Yellow Cherry was found susceptible. No resistant varieties were found.

FRUIT INFECTION

Miss Doidge (2) successfully inoculated unwounded as well as wounded tomato fruits and secured typical scabby lesions when young unwounded fruits were inoculated. In our earlier tests no success attended inoculation of unwounded fruits while infection occurred readily through needle puncture wounds on green fruits but did not result in typical scabby lesions. Higgins (6) reports difficulty in infecting pepper fruits and suggests that there is only a short period early in the development of the fruit when it is susceptible, probably while lenticels are forming. He did not obtain typical scabby lesions from needle puncture inoculations. There is abundant evidence that only young fruits are susceptible.

In our previous account (4) fruit infection through the fruit punctures of stink-bugs and tarnished plant bugs was reported and an opinion was expressed that fruit infection very likely occurs through wounds because of the lack of stomata on tomatoes. The fruit lesions thus obtained were not typical however.

In order to determine the conditions permitting fruit infection and if possible the nature of the white bird's eye spot of tomatoes (Pl. XIV, A, B), two types of insect-proof cages were used in 1921, one of gauze covering the entire plant and the other of celluloid covering a single cluster of fruits. Each cage of the latter type was replaced by a paper bag a few days after it was used.

One to five stink-bugs were caged over each of six clusters of small fruits on July 26 and over each of four clusters on August 4. No inoculum was introduced and upon a total of 23 fruits no bacterial spot nor bird's eye spot developed. Typical stink-bug wounds characterized by a slightly sunken, circular, whitish zone or blotch usually with a black point at the center were abundant on all of the fruits (Pl. XIV, G). Such injuries did not occur on fruits protected from stink-bugs. This type of injury is of common occurrence on tomatoes.

In a parallel series of tests one to eight stink-bugs were caged over each of six clusters of small fruits on July 27 and four clusters on Aug. 4 after the fruits had been atomized with a suspension of the bacteria (A). None of these fruits were over four centimeters in diameter. On Aug. 16, 24 of the 26 fruits showed bacterial spot. There were two types of lesions, one the more typical, small, elevated type (Pl. XIV, D) which develops into a scab, and the other a sunken area of blackened tissue (Pl. XIV, F) about a stink-bug punctures. A section through one of the latter type of lesion is shown in plate XIV, figure E. One bird's eye lesion also developed.

In a third series of tests a number of fruits of various sizes were atomized with a suspension of the bacteria and were caged to exclude all insects. Three fruits between one and 2.5 centimeters in diameter atomized on July 28 showed bacterial spot infection on Aug. 4, which developed into typical scabby lesions. Eight fruits between 0.6 and 2.5 centimeters in diameter, in three clusters, were atomized on Aug. 4 and by Aug. 16 all showed numerous typical lesions of the elevated type and four showed a few bird's eye spots. A number of fruits varying in diameter from 0.6 to 7.5 centimeters on a plant under a large cage were atomized on Aug. 12. Two weeks later typical lesions, in some cases in large numbers, developed on 15 of the 38 fruits atomized and only on fruits that were not more than 2.5 centimeters in diameter when inoculated, of which there were only 17. Therefore 15 out of 17, or 88 per cent, of the fruits atomized when not more than 2.5 centimeters in diameter developed the disease. No lesions developed on six uninoculated fruits held as checks.

Among seven clusters of fruit caged without insects and without inoculation, none developed bacterial spot nor bird's eye spot.

These results show that bacterial spot infection may occur through stink-bug punctures but that typical scabby lesions result from direct infection of very small fruits (diameter less than four centimeters) without insect punctures. The nature of bird's eye spot is not yet proved but it may be a form of bacterial spot.

Since no stomata have been noted on young fruits, the portal of infection is unknown. Evidence of infection through epidermal rifts about the stem end of the fruit has been noted (Pl. XIV, C). Young fruits are thickly covered with club-shaped glandular hairs and long trichomes and as the fruit enlarges these become fewer and more scattered. On old fruits most of these appendages have disappeared leaving only their broken bases. It seems entirely possible that the bacteria may enter through the wounds caused by the breaking off of these delicate epidermal appendages. Groth (5, p. 5) has described minute cuticular cracks in the epidermis through which it also seems possible that the bacteria might enter.

OVERWINTERING

In our previous accounts (3, 4), proof was presented that the organism overwinters on the seed. In fact the organism was found alive on commercial seed 16 months old and had survived 11 months of desiccation on tomato seed in the laboratory. Subsequent cultural tests have shown some of the organisms to be alive on this seed after 16½ months but none after 35 months.

Evidence of overwintering in the field has been obtained. In 1921 a number of volunteer tomato plants came up in a corn field at LaFayette, which had been in tomatoes in 1920. No tomatoes were planted anywhere near this field in 1921. On July 27, 186 of the volunteer tomato plants were examined and two showed bacterial spot infection. Since the disease had been present in this field the year before, this seems to be clear-cut evidence of overwintering of the organism in the soil or in remnants of diseased plants. Consequently, as Miss Doidge (2) has suggested, crop rotation is advisable.

TOMATO SEED DISINFECTION

Seed disinfection in mercuric chloride, 1 to 3,000, for five minutes followed by rinsing has been found effective and practicable as a control measure. For the 1921 crop in Indiana, 1500 pounds of seed were thus treated and, so far as it was possible to determine, there was very little of the disease in the crop, although an absolute control was not obtained. On the other hand the disease was found very prevalent as early as July 13 in certain fields in which the plants were grown from untreated seed. Observations in a canning factory in 1922 on the crop grown from treated seed showed that bacterial spot occurred only very rarely.

SUMMARY

The causal organism of bacterial spot of tomato and pepper should be known as *Bacterium vesicatorium* Doidge.

The optimum temperature for colony growth is near 27° C.

The bacteria endure short periods of freezing in water but are rapidly killed by several days exposure and by alternate thawing and freezing.

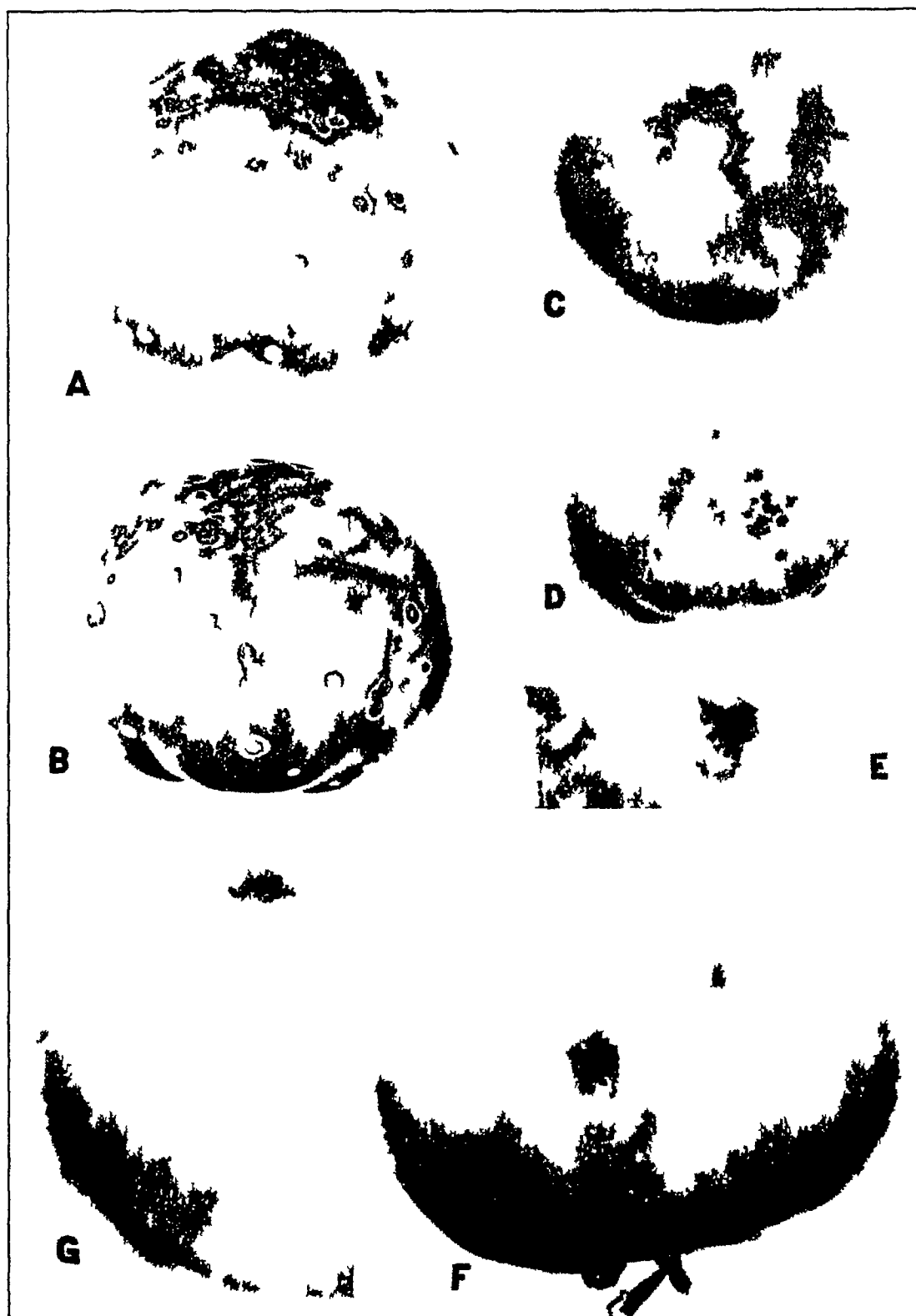
The hosts include tomato (73 varieties), pepper (4 types), potato (leaves), currant tomato, *Solanum nigrum*, *S. dulcamara*, *S. rostratum*, *Datura tatula*, *Physalis minima*, *Lycium chinense*, *L. halimifolium*, *Hyoscyamus aureus*, *H. niger*, and *Nicotiana rustica*.

Fruit infection of tomato may occur through stink-bug punctures but the resultant lesions are not typical.

Typical fruit lesions result from direct infection of very small uninjured fruit.

The organism survived a winter in the field and survived 16½ months desiccation on tomato seed.

Tomato seed disinfection has proved effective and practicable as a control measure.



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DESCRIPTION OF PLATE XIV

Bacterial Spot of Tomato—A. and B. Birds-eye spot of tomato. Cause not proved. Possibly a form of bacterial spot. C. Bacterial spot lesions about stem end of a tomato possibly resulting from infection of small rifts in the cuticle. D. Early stage of bacterial spot. Typical elevated lesions not resulting from infection of insect punctures. E. Section through a stink-bug wound infected with bacterial spot. F. Bacterial spot lesions resulting from infection of stink-bug wounds. G. Stink-bug injury on tomato fruit, not infected. Circular whitish blotches often with a black point at the center.

THE GAMMA STRAIN OF COLLETOTRICHUM LINDEMUTHIANUM (SACC. ET MAGN.) B. ET C.

WALTER H. BURKHOLDER

In 1919 Dr. M. F. Barrus (2) listed over 300 varieties of beans (*Phaseolus vulgaris* L.) and related species, and gave their variation in susceptibility to *Colletotrichum lindemuthianum* (Sacc. et Magn.) B. et C., the fungus causing the anthracnose of this plant. In conducting these inoculation experiments, Barrus gathered together a number of cultures of the pathogene from various sources. These cultures he was able to separate into two groups according to the susceptibility or resistance of certain varieties of beans to them. Two biological strains, therefore, were considered to exist in the species *C. lindemuthianum*. These were designated as alpha and beta. Further isolations of the pathogene made by the writer while working at the Perry Bean Laboratory¹ revealed no other forms. Thus, while it was thought that other strains of this fungus might exist, they were considered rare, and at least in New York State, to be of no importance.

The great majority of the varieties of beans tested by Barrus were susceptible to one or the other of the strains, and many were susceptible to both. A few varieties, however, were resistant to the two strains. Of this latter group the two most important varieties were the Well's Red Kidney and the White Imperial.

The Well's Red Kidney which was brought to the attention of Plant Pathologist by Barrus (1) is immune to strain alpha and highly resistant to strain beta. Since its introduction, it has been grown extensively in New York, and also outside the State. During this time no authentic case has been investigated in which this variety has succumbed to the anthracnose. This appeared to be further evidence of the scarcity of other strains of the pathogene. The White Imperial, the other variety of beans mentioned as resistant to both strains of the anthracnose fungus is resistant to alpha and practically immune to beta. Nevertheless it is not being grown to as great an extent in New York State as the Wells' Red Kidney on account of its lateness which frequently causes it to be prematurely killed by early frosts. Until 1921 this bean likewise continued resistant to the anthracnose as far as inoculation experiments with alpha and beta were concerned, and as far as observations

¹ A laboratory established in 1917 at Perry, New York, for the New York State Bean Investigation, and continued until 1920.

in the field had gone. In that year Barrus (3) writing in regard to the White Imperial states "recently, however, (in 1921) a field of beans said to be White Imperial, the pods of which were severely affected with anthracnose, was observed. It seems not unlikely that strains of the pathogene exist which are capable of infecting even these varieties." No further investigation was made by Barrus of this case, but the address of the grower of the field of White Imperial beans was given to the writer. A quantity of seed was obtained from this field, and in type, it was without doubt a White Imperial. Examination also showed small spots on some of the seeds which appeared to be due to the anthracnose.

During the latter part of January, 1922, petri dish plantings were made from these lesions and a fungus was isolated that proved to be *C. lindemuthianum*. Preliminary inoculation experiments were immediately conducted with this fungus to determine its identity. To obtain spores for these experiments the fungus was grown on bean plugs where it produced great masses of pink spores.¹ Four well-known commercial varieties of beans were used. They were the Wells' Red Kidney, the White Imperial, the White Marrow and the Michigan Robust pea bean. These were selected with the purpose of determining whether the recently isolated fungus was either alpha or beta. It was known that the first two varieties were resistant to both strains of the pathogene, the third variety was resistant to alpha, and the fourth to beta. Of these four varieties only the Michigan Robust proved resistant to the fungus under consideration, so that no definite clue was given to its identity. The Wells' Red Kidney which hitherto had remained resistant to all inoculations of *C. lindemuthianum* was especially susceptible to this fungus. Young seedlings not only showed lesions due to the disease, but were killed within a few days after the symptoms began to appear.

Inasmuch as the Michigan Robust was resistant to the new strain and also to beta, there was the possibility that we were dealing with a vigorous strain of the latter. During the summer of 1919, however, a very virulent strain of beta was isolated from a pod of a *Nova Scotia Marrow*² and tested on 35 varieties of beans. The White Imperial and

¹ This appears to be the usual case with the anthracnose fungus when recently isolated. When grown in culture for a considerable period, a year or even less, the fungus loses this power of fructification to a great extent. Re-isolation, however, will restore this ability and is the only satisfactory method found by the writer for this purpose, although various methods have been proposed by other investigators. This loss of virulence in the pathogene probably varies with the strains since more trouble has been experienced in keeping alpha in a good sporulating condition than with beta.

² A type of White Marrow grown in Nova Scotia, and brought to the attention of the writer by Mr. G. E. Sanders. Under field conditions in New York State it is more or less resistant to the anthracnose.

Wells' Red Kidney remained very resistant to the Nova Scotia strain although varieties susceptible to beta succumbed very rapidly.

To determine further, whether or not the fungus isolated from the White Imperial was a new strain of *C. lindemuthianum*, more than sixty varieties of beans were obtained for inoculation experiments. These experiments were conducted during the spring and summer of 1922 in the following manner. The varieties to be tested were planted in flats in the greenhouse in rows of 25 to 30 individuals. Inoculations were made as soon as possible after germination by spraying the plants with a water suspension of conidia. The flats were then placed in an inclosed chamber, the temperature of which was approximately 18° C. After two days in this chamber the plants were removed and placed in a greenhouse with a temperature of 26° C., or above. Here, at the end of five days or more lesion began to appear. Lauritzen (5) has found that with the anthracnose of the bean very little infection occurs at 27° C., but there is a rapid increase with the lowering of the temperature; excellent infection being obtained as low as 17° C. But the writer is of the opinion that infection having once taken place, the pathogene advances in the tissues more rapidly at higher temperatures. The incubation period is shortened, and the lesions, too, develop to a greater extent.

In all sets of inoculation experiments certain varieties of beans showed excellent infection. The results of these experiments are to be found in table 1. Here, in describing the degree of susceptibility of the varieties of beans to this strain of the pathogene the terminology of Barrus (2) is followed. In his article, these various terms are illustrated with photographs. If table 1 is compared with Barrus's work, it will be seen that the pathogene isolated from the White Imperial agrees very closely with the beta strain of *C. lindemuthianum*. It differs mainly in four varieties. With the Wells' Red Kidney and White Imperial this difference is very pronounced. With the Arlington Red Cranberry and the White Sickle it is less striking, but still evident.

Since the recently isolated strain was so similar in its behavior to beta, further inoculation experiments were desirable before deciding whether or not it was a new strain. There was also the possibility that the seed of the Well's Red Kidney and the White Imperial used in the above experiments varied from the original stock, and for that reason were susceptible. Therefore, 26 strains of the Wells' Red Kidney, 4 strains of the White Imperial, 12 strains of the Yellow Eye, and one each of the Arlington Red Cranberry and the White Sickle were collected. All strains of the Wells' Red Kidney and the Yellow Eye. and two of the

TABLE 1—*Inoculation experiments with the strain of Colletotrichum lindemuthianum isolated from the White Imperial bean.*

Variety	Infection
1 Arlington Red Cranberry.....	good
2 Best of All.....	fair
3 Black Valentine.....	fair
4 Black Valentine.....	fair
5 Canadian Wonder.....	good
6 California Pink.....	very slight
7 Case Knife.....	none
8 Cracker jack Wax.....	good
9 Crease Back.....	none
10 Crystal Wax.....	slight
11 Curries.....	excellent
12 Currie Improved Prolific Tree.....	none
13 Davis Wax.....	excellent
14 Detroit Wax.....	good
15 Eureka.....	excellent
16 Flat Marrow.....	slight
17 French Mohawk.....	good
18 Full Measure.....	good
19 German Black Wax.....	excellent
20 Giant Stringless.....	excellent
21 Golden Eye Wax.....	good
22 Hardy Wax.....	slight
23 Hodson.....	good
24 Isabelle Improved Michigan Pea.....	none
25 Kentucky Wonder.....	none
26 Kentucky Wonder Wax.....	none
27 Lazy Wife.....	good
28 Longfellow.....	good
29 Low Champion.....	good
30 Michigan Robust Pea.....	none
31 Mont d'Or.....	excellent
32 Nova Scotia White Marrow.....	good
33 Old Fashioned Sickle.....	slight
34 Pencil Pods Black Wax.....	excellent
35 Pheasant Eye.....	excellent
36 Prolific Black Wax.....	excellent
37 Red Kidney (Thorburn).....	excellent
38 Red Kidney.....	excellent
39 Refugee.....	excellent
40 Refugee Wax.....	slight
41 Refugee Wax.....	slight
42 Ruby Horticultural.....	excellent
43 Scotia.....	very slight
44 Scotia.....	none
45 Souther Prolific.....	none
46 Sure Crop.....	slight
47 Tennessee Green Pod.....	none

Variety	Infection
48 Turtle Soup.....	none
49 Unrivalled Dwarf Wax.....	excellent
50 Warren.....	fair
51 Wardwell.....	excellent
52 Webber Wax.....	excellent
53 Wells' Red Kidney.....	excellent
54 White Imperial.....	excellent
55 White Kidney.....	excellent
56 White Marrow.....	excellent
57 White Navy (Burpee).....	none
58 White Navy (Harris).....	none
59 White Sickle.....	very slight
60 White Sickle.....	none
61 Wocester Mammoth.....	good
62 Yellow Eye.....	slight
63 Yosemite.....	excellent

White Imperial were obtained from Mr. F. R. Perry of the Hicox-Rumsey Seed Company of Batavia, New York. The Yellow Eyes were originally from Dr. H. H. Love of the Department of Plant Breeding at Cornell, and were pure line strains.

In conducting the inoculation experiments both beta and the newly isolated strain were used. The beta strain came originally from Barrus, and excellent infection had been obtained when susceptible varieties of beans were inoculated with it. Nevertheless, to make this culture more comparable with the new strain, some White Marrows were infected and a re-isolation made. This re-isolated culture was then used in the following experiments. The inoculations were conducted in the same manner as previously described. Corresponding flats were inoculated at the same time with beta and the new strain. The data are set forth in table 2.

With the Wells' Red Kidney it will be noted that the new strain gave excellent infection in the majority of cases, good in a lesser number, fair in two cases, and slight in one. On the other hand, the beta strain caused no infection in the majority of cases, slight in one, fair in two, good in one, and excellent in one. One significant point in this experiment-test should be noticed, and that is, that those strains showing a slight susceptibility to beta frequently exhibit, a slight resistance to the White Imperial strain. This is the condition in numbers, 3, 7, 12, 18, and 21. It cannot be argued then, that the new strain is only a more virulent form of beta. In the Yellow Eye, too, it may be observed that some of the pure lines are more resistant to the new strain than to beta. As in the majority of Barrus's inoculations the Arlington Red Cranberry was resistant to the beta strain, but excellent infection was

TABLE 2—*Inoculation experiments with the beta strain and the White Imperial strain of C. lindemuthianum.*

No. ¹		Beta Strain	Infection White Imperial strain
Wells' Red Kidney			
2.	none.....	excellent	
3.....	slight.	good	
4.....	none.....	excellent	
5.	none.....	excellent	
7.....	fair.....	good	
8.....	none.....	excellent	
9	none.....	excellent	
10.....	none.	excellent	
11....	none.	excellent	
12	good	slight	
13....	none..	excellent	
14	none	excellent	
17.....	none..	excellent	
18	fair.	good	
21....	excellent.....	good	
29.....	none.....	excellent	
31	none.....	excellent	
32.....	none	fair	
37.....	none	good	
38.....	none	excellent	
51.....	none.....	excellent	
52.....	none.....	excellent	
53.....	none	fair	
54.....	none.....	good	
55	none.....	excellent	
56.....	none.....	good	
White Imperial			
1.....	none.....	good	
2	none.....	good	
3.....	none.....	good	
4.....	none.....	good	
Yellow Eye			
2-7-28-30-7.....	slight.....	none	
5-68-45-1-3.....	slight.....	none	
17-541-8-5-3.....	good.....	fair	
17-541-8-5-44.....	good.....	slight	
17-541-12-1-6.....	fair.	none	
20-560-1-3-1.....	slight.....	fair	
20-560-1-3-11.....	slight	fair	
26-474-6-5-12.....	slight	fair	
26-474-6-5-27.....	slight.....	fair	
26-478-27-13-6.....	slight.....	fair	
26-478-27-13-22.....	slight.....	fair	
26-478-29-20-8.....	slight.....	fair	
26-478-29-20-25.....	slight.....	fair	
Arlington Red Cranberry.....	very slight.....	excellent	
White Sickle.....	very slight.	none	

¹ The pedigree numbers of the Wells' Red Kidney and the Yellow Eye are those used by Mr. Perry and Dr. Love.

obtained on this variety in the experiments being reported upon, with the White Imperial fungus. This fact also seems to indicate that the recently isolated fungus is a new strain. On the other hand, the White Sickle was resistant to both pathogenes and was evidently a different strain of that variety of bean from the one used by Barrus in his experiments.

Much of the work done at the Perry Bean Laboratory to control the anthracnose has been in breeding strains of beans resistant to the disease. Dr. G. P. McRostie (6 and 7) and the writer (4) have shown that resistance to alpha and beta respectively is dominant and governed by a single factor. Just how the resistant to the third strain of *C. lindemuthianum* would react in hybridization was an important point to be considered in further breeding experiments. A small number of seed from the F₁ plants of crosses between the Wells' Red Kidney and the Michigan Robust pea bean were on hand. These were planted in flats and inoculated with the third strain. The data which were collected and tabulated on July 26, 1922 may be found in table 3. The number of F₂

TABLE 3—Results of the inoculation of F₂ plants of a cross between Wells' Red Kidney and Michigan Robust pea bean with the White Imperial strain of *C. lindemuthianum*.

Pedigree number	Resistant	Susceptible
402	82	23
403	57	23
404	9	2
405	34	8
406	27	9
Totals	209	65
Expected number	204	68

individuals is small, but with the close approximation to a three to one ratio, there seems to be no doubt that resistance is dominant and governed by a single factor. In this respect it agrees with the resistance to both alpha and beta. It should be observed that in this experiment the Wells' Red Kidney was used as the susceptible parent. In the hybridization experiments previously recorded, Burkholder (4) and McRostie (6 and 7), this variety had always been used as the resistant parent.

From the foregoing experiments the strain of *C. lindemuthianum* isolated from the White Imperial appears to be distinct from either alpha or beta. It is true that it is closely related to the latter, but the inoculation experiments on the Wells' Red Kidney and the Yellow Eye show that it is not merely a more virulent form. The hybridization experiments too, lend proof to this point since the susceptible parent in the experiment was resistant to the beta strain of the pathogene.

In speculating as to the origin of this new strain, the writer considers it is not improbable that it arose from the beta strain as a mutation. The close relationship of the two strains as demonstrated in table 1 points toward this possibility. It is also known that the beta strain under favorable circumstances will produce, at times, fair sized lesions on young seedlings of the Wells' Red Kidney, so the further step to the new strain would not be great. Such a case not unlikely was the beginning of the third strain. Moreover, the county in which this new strain was found, is one in which the Wells' Red Kidney is grown extensively, and under these circumstances chance would be given for its development. All things considered, however, the writer feels justified in regarding the fungus isolated from the White Imperial sufficiently removed from beta to be considered a new strain of *C. lindemuthianum*, and for this the Greek letter *gamma* is given to correspond with the alpha, and beta strains.

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TRYPANOSOME-LIKE BODIES IN SOLANACEOUS PLANTS

J. E. KOTILA AND G. H. COONS

Immediately following the recent report by Nelson (1) of the occurrence of protozoa in bean, clover, and tomato plants affected with mosaic and potato plants affected with leaf roll, investigations were undertaken to ascertain whether the same conditions obtained in potato plants affected with other degenerative diseases.

Diseased material from plants showing severe symptoms of potato mosaic, streak and leaf roll was killed and fixed with both chrome-acetic and Zenker's solutions. Longitudinal sections seven microns thick were cut and stained with Haidenhain's haematoxylin, the sections being mordanted 4 to 6 hours, stained for 12 hours and restained until most of the haematoxylin had been removed from the cell nuclei. The destaining process seldom required more than two minutes in the 4 per cent iron alum mordant which was used.

Cell inclusions resembling the trypanosome-like bodies described by Nelson were found in phloem cells of potato plants affected with mosaic and streak. Likewise, similar bodies were found in the sections from plants affected with leaf roll.

Trypanosome-like bodies having been found in the material from the potato plants affected with the various diseases, the studies were extended to other Solanaceous hosts affected with mosaic. Midribs and lateral veins of leaves of mosaic tobacco and petunia plants were killed, fixed and stained as described for potato. No difficulty was experienced in locating in the phloem cells, inclusions similar to those found in the diseased potato plants.

As a control upon the study of diseased material, similar work was done with material from healthy potatoes and tomatoes. The stock from which the healthy potato material was taken was indexed¹ during the winter of 1921-1922, found to be healthy, and planted in an isolated plot in a sunflower field during the 1922 growing season. No mosaic or leaf roll appeared in the plot during that summer. Some of the progeny of this healthy stock was indexed again during the present winter, it being planted in a greenhouse in which indexing of potatoes was being carried on. Aphids were extremely scarce on the potatoes in the greenhouse and none were observed on the plants which were selected for study. No signs of either mosaic or leaf roll were observed on any of the plants which had reached the height of eight inches at

¹ In this work, an eye of a tuber was sprouted and grown in the greenhouse to approximately 10 inches in height, and evidences of disease looked for. The tubers from which any aberrant plants developed were rejected.

the time material was cut and fixed. The stock of potatoes studied was of the Bliss Triumph variety, upon which symptoms of either mosaic or leaf roll can easily and promptly be recognized.

The tomato material was obtained by planting disinfected (chlorazine 1 per cent) seed on agar in petri dishes, and after germination by transferring the seedlings to test tubes containing Shive's nutrient agar. Material was taken for study when the seedlings were approximately one and a half inches tall. The test tubes containing the seedlings were kept in test tube racks in a window of the laboratory and being plugged with cotton there was no possibility of contamination by aphids. The only possible source of disease was in the seed itself. Allard (2) and Gardner and Kendrick (3) contend that tomato mosaic is not transmitted through seed and base their contentions on negative results obtained from planting seed and growing thousands of tomato plants under controlled conditions. Gardner and Kendrick (3) state "In the light of all the available evidence, there appears to be no indication whatever that tomato mosaic is transferred through the seed."

It is believed that this material studied was healthy. Stained sections from both potato and tomato plants revealed cell inclusions in the phloem elements which were similar in size and form to those found in severely diseased material and which were equally abundant.

Although the cell inclusions observed were trypanosome-like in shape, they did not possess any degree of uniformity. Great variation in size was found in the bodies from both diseased and healthy material. All attempts to show definite structure by using such protozoan stains as Wright's, Romanowski's, tetrachrome, etc., have resulted in failure, the bodies staining a uniform blue. Likewise it has been impossible to demonstrate motility by means of thin sections of fresh material from either diseased or healthy plants or in extracted juice of such plants.

From the study which has been made of both diseased and healthy plants it is believed that the correlation of the trypanosome-like bodies described by Nelson with mosaic and leaf roll has not been proved and further investigations are necessary.

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MICHIGAN AGRICULTURAL COLLEGE,
MARCH 1923.

INTRACELLULAR BODIES IN THE PHLOEM TISSUE OF CERTAIN PLANTS AND THEIR BEARING ON THE MOSAIC PROBLEM

S. P. DOOLITTLE AND H. H. MCKINNEY

WITH PLATE XV

In connection with investigations of mosaic diseases the writers have studied large amounts of phloem tissue of both mosaic and mosaic-free plants.

Studies have been made of stem and petiole phloem elements of mosaic-free and mosaic-infected navy beans, tomatoes, cucumbers, and red clover and of healthy garden peas, sweet peas, and alfalfa. In most cases free-hand razor sections of fresh material were studied in comparison with material fixed, embedded, sectioned, and stained by the usual methods. In these studies certain intracellular bodies have been found in the phloem of both mosaic and mosaic-free plants. In view of the very close similarity of these bodies to those which Nelson (2, 3) recently described as protozoa in the phloem of certain mosaic plants, it seems desirable to publish some of our recent results.

The mosaic-free bean and tomato plants studied by the writers were grown in an isolated greenhouse at the University of Wisconsin, in which no mosaic plants were found, either before or after the time the material for study was taken, even though light and temperature conditions were favorable for the development of mosaic symptoms. In fact, no mosaic-infected tomato plants were found in any of the University greenhouses. Furthermore, healthy tomato plants inoculated with juice from the healthy plants sectioned developed no mosaic, while similar healthy plants inoculated at the same time from the mosaic plants studied, all developed the disease. The mosaic-free beans used were grown from seed of both mosaic-resistant and mosaic-susceptible varieties taken from mosaic-free plants.¹

The mosaic-free cucumbers were grown at Madison, Wis., under cheesecloth cages, which have proven effective in preventing mosaic infection by excluding the insect carriers. The mosaic-free red clover plants and healthy garden peas, sweet peas, and alfalfa were grown in the University greenhouses from healthy seed.

¹ The writers are indebted to Mr. W. J. Evans, graduate student in the Department of Plant Pathology, University of Wisconsin, for bean seed and plants with which he was working in connection with his mosaic resistance studies. They are also indebted to Dr. F. R. Jones and Mr. John Monteith, Jr., for pea and clover material, respectively.

The mosaic-infected tomato plants were obtained in a commercial greenhouse in South Madison, Wis. The mosaic-infected bean and clover plants were grown in the University greenhouses at Madison. The mosaic-infected cucumbers studied were grown in fields and greenhouses at Madison.

In mosaic-free and mosaic-infected bean plants, three to six weeks old, the bodies were found both in unstained, freehand razor sections of fresh material cut longitudinally and in stained, longitudinal sections cut 9-12 microns thick from material killed in various fixatives, embedded and stained either by Flemming's triple stain or by Heidenhain's iron-alum haematoxylin. The bodies were found singly in the sieve-tube and adjoining cells oriented longitudinally. They were of various shapes from irregularly ovoid to narrowly ellipsoid and usually had one or more slender strands extending from each end. In some cases, these strands extended to the ends of the cells, and in others, limited distances from the bodies (Pl. XV-B). While the general form of some of these bodies may suggest that of certain protozoa, the characteristic structural details of protozoa, such as nuclei, blepharoplasts and rhizoplasts, were not found when the material was stained with Heidenhain's iron-alum haematoxylin and carefully destained, or when stained with Flemming's triple either of which stains should show these internal structures if present. In free-hand razor sections of fresh material, no active motion of the intracellular bodies was noted, such as might suggest that of a protozoan.

In mosaic-free and mosaic-infected red clover, as well as in healthy garden peas, sweet peas and alfalfa, intracellular bodies have been found which, in general, are similar to those found in the bean, except that the terminal strands have been noted only occasionally in clover and alfalfa and not at all in garden peas or sweet peas.

While the writers have not yet made an exhaustive search for literature bearing on these intracellular bodies, it is of especial interest to note that in 1891 Strasburger (4) reported finding, in presumably healthy plants of black locust (*Robinia pseudoacacia*), certain bodies which resemble, in many respects, the bodies found by the writers in beans, clover, garden peas, and sweet peas (Pl. XV-A).

The pertinent portion of the Strasburger (4) article has been translated as follows:

"The mature sieve-tube cells contain ellipsoidal bodies (Pl. III, Fig. 4).¹

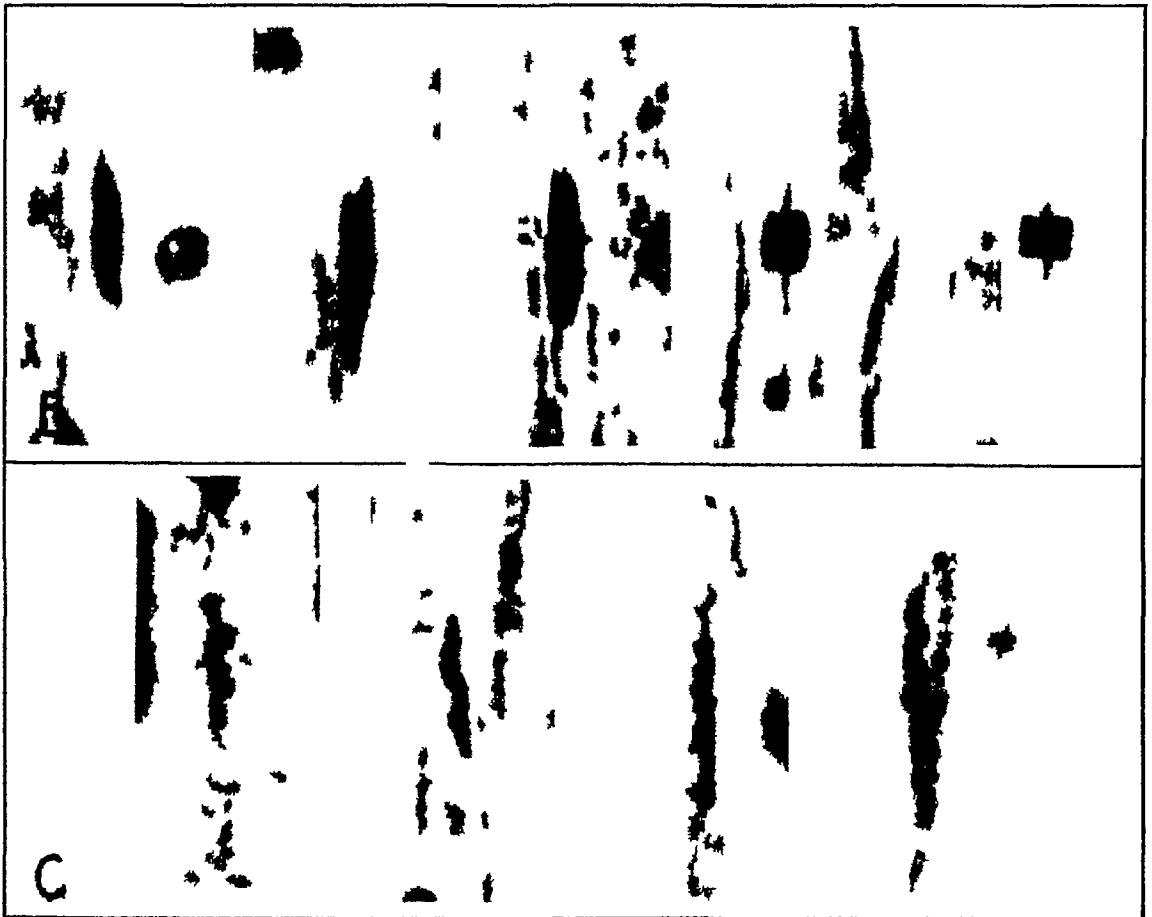
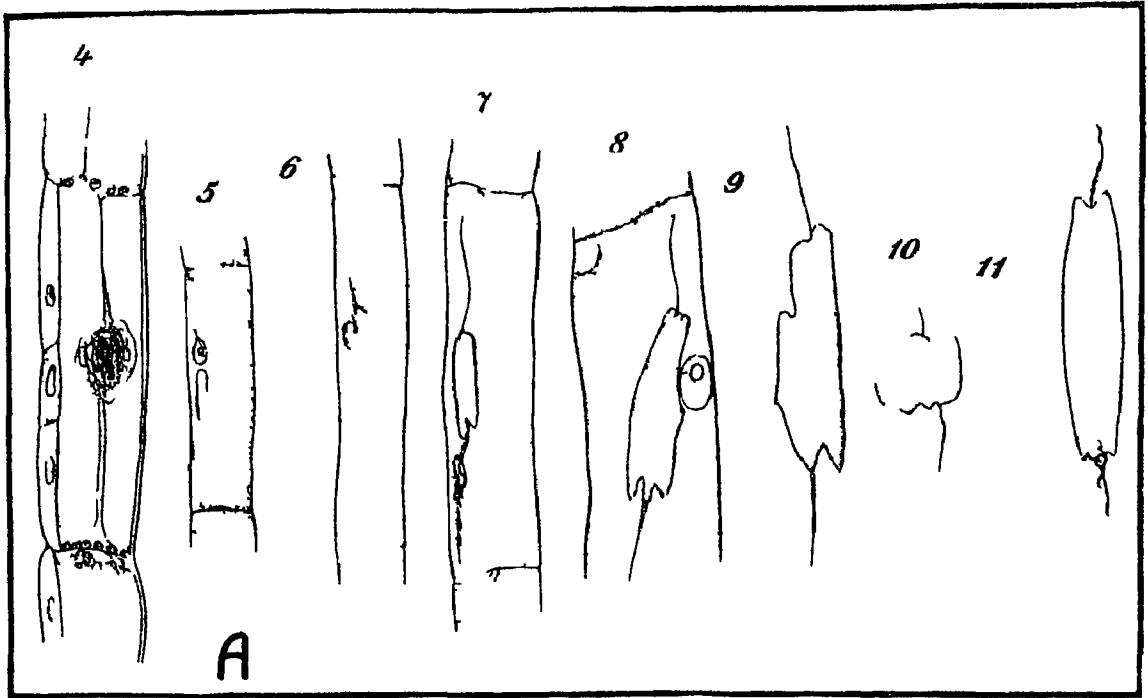
In an alcoholic preparation there is to be seen in each element a strongly refractive lump which gives the customary reaction of the sieve-tube slime, but which generally appears to be suspended in the middle of the cell. The suspension occurs by means

¹ Strasburger's Plate III, figs. 4-11 are reproduced in our plate—A.

of thin threads which lead from ends of the slime-body toward the sieve-plates. This thread becomes increasingly attenuated until it reaches the sieve plate, where, however, it often widens. If the slime body lies against one or the other sieve-plate, it sends a single thread to the other sieve-plate. The suspended slime-body has a somewhat ellipsoidal or angular polygonal shape. * * * The slime-body colors a yellow-brown with iodine although the thread takes the same color, only exceedingly faintly. The slime-body similarly reacts intensively in Millon's reagent and the threads weakly. Ontogeny shows that the bodies arise from the primordial protoplasm of the cell. It shows at the time of its formation, chiefly a spindle (Pl. III, Fig. 5-7), seldom a barrel-shaped (Pl. III, Fig. 10) form. As soon as the slime body reaches a certain size the thread leading from the body to the sieve-plates becomes visible. This thread also is differentiated from the inner portion of the peripheral protoplasmic layer. The slime-bodies swell into an ellipsoidal form (Pl. III, Fig. 7-9), and together with their suspension threads, pull away from the peripheral layer of protoplasm. In the meantime the nucleus shrinks and the protoplasmic layer is very decidedly reduced. The protoplasm contracts in the young sieve-tubes but in old ones fails to contract and is therefore not always readily demonstrable. It often happens that more than one suspension thread may lead from the same end of a slime-body to one or the other of the sieve-plates. Less often the slime-body arises in contact with the sieve-plate. One finds them commonly at the sieve-plates but this is usually a result of the cutting of the stem sections and the influence of the alcohol. That the suspended slime-body cannot be considered an artifact is easily demonstrated in fresh material in which the somewhat refractive slime-body as well as its threads can be readily recognized."

Haberlandt (1) states that these slime-bodies occur generally in the Leguminosae but do not occur in the Cucurbitaceae. Recent as well as earlier studies by one of the writers (Doolittle) are in accord with this statement, as hundreds of longitudinal sections made from both mosaic and mosaic-free cucurbit stems and petioles have never revealed such bodies in the phloem tissues.

In the case of the tomato, the intracellular bodies, interpreted by Nelson as trypanosome-like protozoa, apparently do not occur as regularly as the bodies which are found in the bean, but they seem to be fully as abundant in the mosaic-free as in the mosaic tissues (Pl. XV-C). Careful staining and destaining of these bodies by the methods used in studying the bodies in the bean has failed to reveal structure typical of trypanosomes or other protozoa. Examinations of the tissues of both mosaic and mosaic-free plants show that the cytoplasm of the phloem cells frequently collects in lightly stained, long or short, irregular, spiral, wavy forms and it is possible to find masses which show all stages between this cytoplasmic condition and the deeply stained forms which seem to be identical with the bodies described as protozoa by Nelson. The writers have looked for a possible undulating membrane, a characteristic of the trypanosomes, but none has been found. In some cases the outer edges stain lighter than the central portion of the bodies, but this could easily be due to wrinkled or folded host cytoplasm.



INTRACELLULAR BODIES OF MOSAIC VIRUS PLANTS

While it is recognized that Strasburger might have been working with mosaic plants, this seems improbable. The possibility of infection in the case of the plants which the writers have considered mosaic-free is extremely slight, as they were grown under conditions which assured unusual protection from infection by means of insects or other agencies and which would not favor a masking of symptoms.

As the bodies described by Nelson in beans, clover, and tomato have been found by the writers in mosaic-free plants,¹ and as they cannot be distinguished from what seem to be normal constituents of the cells, further work will be necessary before it can be said definitely that protozoa are present in plants affected by mosaic diseases.

¹ Since preparing this manuscript, Dr. E. A. Bessey, of the Department of Botany, Michigan Agricultural College, who is familiar with Nelson's material, has seen the material studied by the writers and has stated that the bodies with which they are working appear to be identical with those described by Nelson.

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LEGENDS FOR PLATE XV

- A. Copy of figs. 4-11 from Pl. III of Strasburger paper (4), a portion of which is translated in text, showing slime-bodies in the sieve-tubes of *Robinia pseudoacacia*. Translation of original legends:
Fig. 4. Mature sieve tube elements with companion cells; in the interior of the sieve-tube the slime-body suspended on a thread. (× 240.)
Figs. 5, 6, & 7. Young sieve-tube elements which show the ontogeny of the slime-body in the peripheral protoplasmic layer. (× 240.)
Figs. 9, 10, & 11. Slime-bodies more highly magnified. (× 540.)
- B. Various bodies found by the writers in the sieve-tubes of mosaic-free navy bean plants. (× 1144.)
- C. Various bodies found in the phloem-tissue of mosaic-free tomato plants. (× 1144.)

NELSON'S SPIRAL BODIES IN TOMATO MOSAIC NOT PROTOZOANS

CHARLES A. KOFOID, HENRY H. P. SEVERIN AND OLIVE SWEZY

Since the publication of Nelson's account¹ of his evidence for the occurrence in certain mosaic diseases of plants of protozoans resembling trypanosomes, we have been able to examine both normal tomato plants and those with typical mosaic. These plant tissues we fixed in hot Schaudinn's fluid and stained with the refinements of protozoological technique in iron haematoxylin. We find structures in both the normal and diseased tissues thus prepared which belong clearly to the same category as the structures described and figured by Nelson. We are wholly unable to agree that these are trypanosomes and find nothing convincing that they are of a protozoan nature.

They are not trypanosomes, for they have no undulating membrane, no flagellum with a definite, clear-cut, marginal fibril arising from a centrosome. Neither is there a parabasal body, the so-called kinetonucleus of earlier literature and textbooks, near the end of the fibril and joined to the centrosome by the parabasal rhizoplast. Furthermore, we are unable to agree that the spiral form of these bodies, which are often twisted in a total spiral, is characteristic either of trypanosomes or of any known protozoans. Its modifications are too strangely varied to fall into any known category of the morphological aspects of the protozoa. In addition, we can not agree that a nucleus of flagellate type with a nuclear membrane, a karyosome, and peripheral chromatin, such as might be expected in an organism with trypanosome affinities, or, indeed, of any known protozoan type, is present in these bodies. Nelson's figures do not demonstrate it to us and we can not agree that the darker aggregates sometimes demonstrable in the spiral bodies have either the constancy of structure, the type of variability, or the evidences of mitosis to be found in protozoan nuclei.

These spiral bodies of tomato mosaic do not belong in the same category with the clearly established trypanosomes of the latex of Euphorbiaceous plants, which bear all the morphological tokens of their protozoan and trypanosome affinities.

We did not see at any time any evidence of motility on the part of these spiral bodies, when observed in the expressed juices of the tomato plant. There was no movement of an undulating nature, no lashing

¹ Nelson, Ray The occurrence of protozoa in plants affected with mosaic and related diseases. Mich. Agr. Exp. Sta. Tech. Bul. 58: 1-30, 18 figs. 1922.

of a flagellum, no darting across the field of the microscope such as characterize the activities of motile flagellates. Occasionally reagent bottles, notably those containnig physiological salt solution, which are in use in the laboratory, become contaminated with a species of *Bodo*, a small, rapidly-moving flagellate. These might appear in preparations made up with such contaminated solutions and deceive the observer as to their origin.

Finally, we find the same, or at least similar structures in comparable locations in supposedly healthy and evidently mosaic-free seedling tomato plants, as well as in the diseased phloem of older plants. The only difference noted between the spiral bodies from the diseased and supposedly healthy plants is that the former are larger in most instances observed in our material. We are unable to state whether or not this difference bears any relation to either age or to disease of the plant. This occurrence of the spiral bodies in both supposedly healthy and obviously diseased plants adds to our skepticism as to their protozoan affinities and as to the possibility that they are flagellates from the digestive tract of plant feeding Hemiptera. The peculiar relations of these spiral structures in their elongated, trumpet-shaped phase to the sieve plates in some instances, their relative homogeneity, and their spiral structure, lead us to believe that they are dexiotropic cell contents of an albuminoid nature.

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SLIME BODIES OF ROBINIA PSEUDO-ACACIA L.

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WITH PLATE XVI

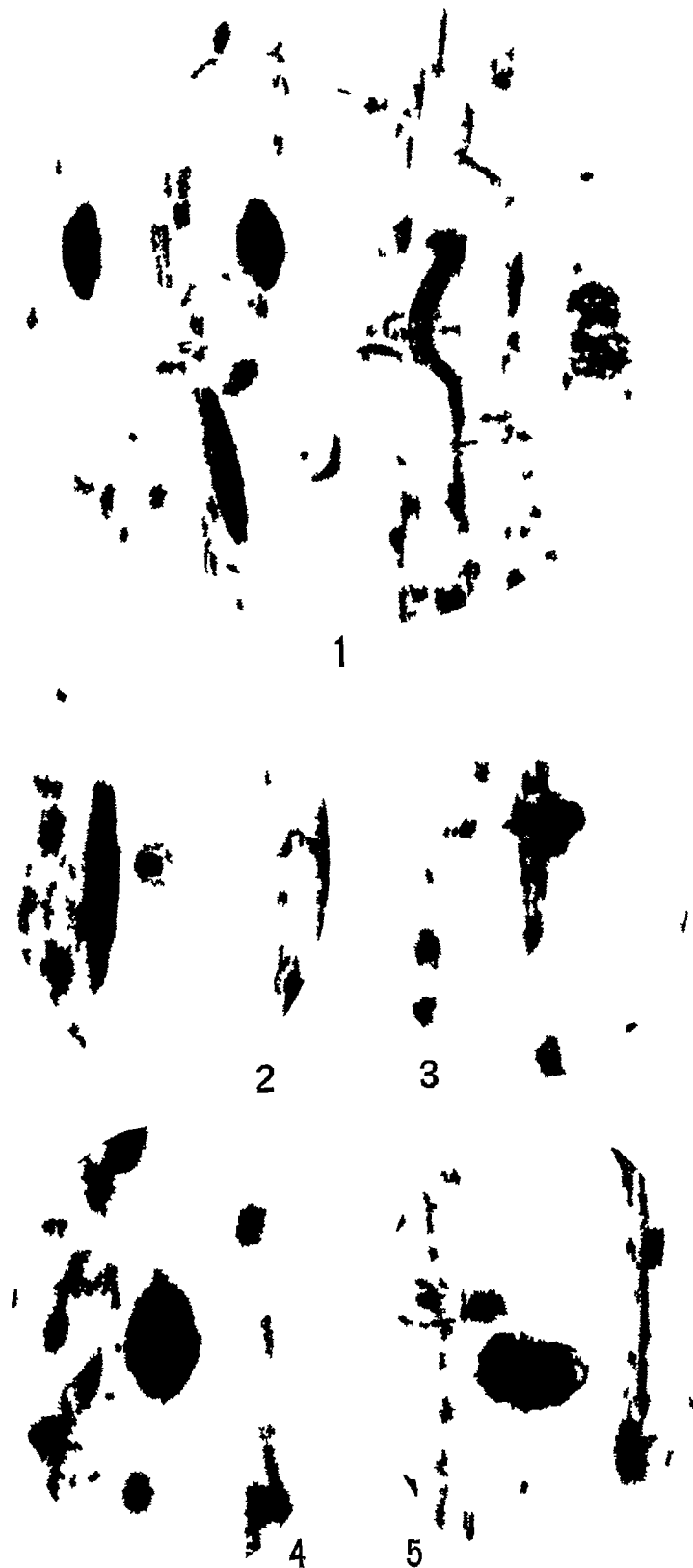
In connection with my work upon the cytology and histology of the lateral meristem, I have had occasion to collect carefully killed and fixed specimens of the cambium and phloem of the stem of *Robinia Pseudo-Acacia* L. As shown by Strasburger¹ (1891), this tree is characterized by having curious "slime-bodies" (Schleimklumpen) in its sieve tubes. The "slime-bodies," which stain intensively in Millon's reagent, vary greatly in size and shape during different stages in the differentiation of the sieve tubes. At an early stage, they are small, slender, spindle-shaped structures, but later they enlarge, assuming the various forms illustrated in plate XVI, figures 1-5. The larger, spindle-shaped masses, which frequently tend to be bifurcated at the ends (Pl. XVI, fig. 3), or more or less deeply cleft longitudinally, are held in place by slender threads attached at the ends of the spindle. As the "slime-bodies" enlarge laterally, these strands become thicker and more conspicuous (Pl. XVI, figs. 4 and 5).

That the organisms, described and figured by Nelson² in the phloem of bean and clover affected with mosaic are "slime-bodies," such as occur normally in the sieve tubes of *Robinia* and other Leguminosae, seems certain. Nelson emphasizes the extreme polymorphism in the life cycle of the supposed flagellates and cites the following principal forms:

(1) Large, elongated, biflagellate, tapering, or cigar-shaped individuals with the form of the body resembling organisms belonging to the genus *Leptomonas*, the flagella being attached at opposite poles. This is the most common type. (2) Ovaliform biflagellates of the same type. (3) Deep-staining, slightly elongated bodies, surrounded by a lighter envelope, with flagella running down to the centrally stained portion and projecting from each end of this destained envelope. Individuals of this type may be almost spherical and occur in pairs in the same cell. (4) Very small, elongated flagellates, less than 0.5 micron in diameter and similar to type 1. These are common in sieve and parenchyma cells and probably represent very young forms of type 1. (5) Deeply stained oval bodies with degenerate flagella and occasional remnants of an outer envelope. These may be encysted forms.

¹ Strasburger, Edward. *Histologische Beiträge*, Heft 3: 193-194. 1891.

² Nelson, Ray. The occurrence of protozoa in plants affected with mosaic and related diseases. *Mich. Agr. Exp. Sta. Tech. Bull.* 58, p. 1-30, 18 figs., 1922.



SLIME BODIES OF ROBINIA PSEUDO ACACIA

The "slime-bodies" of *Robinia Pseudo-Acacia* take on all of these forms during various stages in the differentiation of the phloem. Types 1, 2, and 4 have already been referred to. The forms with a "destained" outer envelope occur commonly in nearly mature sieve tubes. The paired condition is illustrated in plate XVI, figure 1 (extreme right) Bifurcated or cleft "slime-bodies," suggesting stages in longitudinal fission, are of frequent occurrence. During the later stages in the enlargement and modification of the "slime-bodies," the sieve tube nuclei degenerate, as they do normally in most Gymnosperms and Angiosperms. The "slime-bodies" may, in certain cases, be in contact with the nuclei (Pl. XVI, fig. 3). Furthermore, although the smaller, spindle-shaped "slime-bodies" are relatively homogeneous, they may contain one or more deeply staining granules.

In discussing the identity of the structures described by Nelson, it should be emphasized that those figured by the writer are larger owing to the fact that they were obtained from stems of large trees. The cambial initials and their derivatives are larger in old robust stems than in young slender shoots, and the larger sieve tube elements tend to have larger "slime-bodies."

The movements recorded by Nelson in freshly cut sections of living material mounted in boiled water are to be expected. In removing the sections from the plant, the stresses and strains in the various tissues are changed, and the osmotic and other equilibria are disturbed upon emersion in water. That the "slime-bodies," attached as they are by long slender threads, should remain perfectly stationary during subsequent readjustments would be remarkable, even if the liquid or semi-liquid contents of the cells had no circulatory movements.

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DESCRIPTION OF PLATE XVI

Fig. 1. *Robinia Pseudo-Acacia* L. Tangential longitudinal section of phloem of stem of an old tree, showing spindle-shaped, curved and ovaliform "slime-bodies." $\times 416$.

Fig. 2. *Robinia Pseudo-Acacia* L. Portion of sieve tube in tangential longitudinal section, showing "flagellate," spindle-shaped "slime-body" and nucleus with deeply stained nucleolus. $\times 690$.

Fig. 3. *The Same*. Portion of sieve tube in tangential longitudinal section, showing spindle-shaped, bifurcated "slime-body" in contact with nucleus. $\times 690$.

Fig. 4. *The Same*. Portion of sieve tube in tangential longitudinal section, showing "biflagellate," ovaliform "slime-body." $\times 690$.

Fig. 5. *The Same*. Portion of sieve tube in tangential longitudinal section, showing "triflagellate," transversely expanded "slime-body." $\times 690$.

FURTHER NOTES ON THE RELATION OF THE SPORE LOAD TO THE PER CENT OF STINKING SMUT APPEAR- ING IN THE CROP

F. D. HEALD AND L. W. BOYLE

In a previous contribution¹ the results were given of a study of the relation of spore-load, or the quantity of spores carried by each grain, to the percent of stinking smut appearing in the crop, for both naturally and artificially smutted seed. It was pointed out that the determination of the spore-load offered a method of predicting the percent of smut that would appear in the crop under optimum conditions provided no seed disinfection should be practiced, thus making possible a rational selection of wheat for seed purposes and also indicating whether seed treatment in any given case would be a paying procedure. Some additional results from experiments in progress at the time of writing the first article, are now available and will be presented, since it is not planned to carry out any further tests bearing on this problem.

In the spring of 1921 tests were made with the same two varieties, Marquis and Jenkins Club, as used in the previous experiments, with the addition of Early Baart, Hybrid 143, Little Club and Bluestem. One-hundred-gram lots of seed of each variety were smutted by shaking in a glass bottle with the respective amounts of smut, for lots carrying 0.04 to 3 grams. For lots of seed carrying 0.02 grams or less of smut, distribution of the spores was accomplished by making a suspension in water and spraying them over the grain with an atomizer. It was felt that the latter method insured a better distribution of the spores when such small quantities of smut were being used. The smut was distinctly evident by the coloration of the "brush" in all samples down to and including 0.08 grams of powdered smut to each 100 grams of seed, but was not visible except on white grains when less than 0.04 grams of smut was used. From the table 1 it may be noted that grain may be carrying several thousand smut spores per berry and still show no evidence of smut by naked eye inspection.

Two rod-rows of each lot of seed were planted by hand on the same day, using as nearly as possible uniform depth and spacing of seed, so that the different lots would be directly comparable. Plant and head counts were made at harvest time to determine the percent of smut in the different plots. Very soon after seeding time spore counts were

¹ Heald, F. D. The relation of spore load to the per cent of stinking smut appearing in the crop. *Phytopathology* 11: 269-278. 1921.

made for each lot of seed using the Levy counting cell and mechanical stage and following the same method as in the previous work.¹

The spore-load for the different varieties is given in the appended table (Table 1). The maximum number of spores per grain was 278,616 for

TABLE 1—*The number of spores per grain, using various weights of smut per 100 grams of seed.*

Weight of smut per 100 grams seed	Early Baart	Marquis	Jenkins Club	Hybrid 143	Little Club	Bluestem
3 grms	263,973	263,116	152,441 (?)	163,675	237,619	278,616
2	181,480	210,626		120,016	164,798	209,872
1	84,033	100,402	75,795	61,297	81,578	102,440
0.8	63,689	76,864	73,671	50,710	68,224	77,698
0.4	27,539	39,491	33,404	28,912	31,716	40,331
0.25	17,909	19,677	18,678	17,659	24,673	23,337
0.1	6,926	6,843	6,510	5,429	12,521	7,925
0.08*	5,241	5,430	5,262	5,590	4,992	6,635
0.04**	1,976	1,997	2,371	1,716	1,664	3,161
0.02	1,019	1,061		499	1,144	1,289
0.01	499	561	1,227	644	686	624
0.005	312	478	936	457	416	416
0.00	104	21	790	228	229	0

Bluestem with three grams of smut per 100 grams of seed, while the lightest spore load varied from 312 to 936 per gram with 0.005 grams of smut per 100 grams of seed. The spore counts show a varying capacity of the different varieties for holding the smut powder, as may be noted by the smaller number in Jenkins Club and Hybrid 143. This variation may be due to the size of individual grains, the length and thickness of the brush, and other surface characters. It should be noted that all the unsmutted samples showed smut with the exception of Bluestem, which appeared to be smut-free.

The percent of smutted plants is given in table 2. With the exception of certain fluctuations which might be expected there was a gradual reduction in the percent of smutted plants with the diminished spore-loads. It may be noted that maximum smutting was obtained with one gram of smut per 100 grams of seed with Early Baart, Jenkins' Club and Little Club, while 2 grams of smut were required to produce maximum smutting with Marquis, Hybrid 143 and Bluestem. The portion of the table giving the results with spore-loads invisible or barely visible to the naked eye (.08 grams or less) is of special interest

¹ Loc. cit. p. 272-273.

* Smut distinctly visible to naked eye.

** Barely visible on white grains, but not on dark grains like Marquis.

as indicating the amount of smut which might be expected if visibly clean seed is selected for planting. With such samples the percent of

TABLE 2—*Per cent of smutted plants for different degrees of smutting.*

Weight of smut per 100 grams seed	Early Baart	Marquis	Jenkins Club	Hybrid 143	Little Club	Bluestem
3 grams	53.6	6.5	63.6	68.4	29.2	44.5
2	50.2	17.4	64.8	78.7	33.0	53.2
1	70.0	11.0	72.0	42.4	38.0	47.5
0.8	58.9	9.0	57.4	37.9	24.5	37.5
0.4	4.6	0.0	11.1	15.5	6.3	17.0
0.25	28.3	0.5	2.9	9.8	1.0	3.0
0.1	0.5	0.0	4.0	5.3	0.0	1.8
0.08	1.6	0.0	1.8	1.6	0.0	1.0
0.04	0.5	0.0	1.0	0.0	0.8	1.9
0.02	1.6	0.8	13.6	4.7	4.2	3.9
0.01	2.8	1.7	6.9	6.9	1.0	2.9
0.005	3.9	0.0	5.6	2.2	1.0	2.1
0.0	0.0	0.0	1.2	0.0	0.0	0.0

smut varied from 0 to 6.9 per cent with a single sample showing 13.6 per cent. Attention should be called to the pronounced jump in per cent of smut in all lots with spore-load of 0.02 grams per 100 grams of seed, and the gradual decrease again with the smaller spore-loads. This abrupt change can be explained only by the method of treating and distributing the spores. If all spores had been dusted on the grain there is every reason to believe that there would have been a gradual drop in the per cent of smut to the end of each series. In the previous paper it was shown that the per cent of smut appearing in the naturally smutted spring wheat is less than would be indicated by the spore-load as based on plantings of artificially smutted samples, this being due presumably to the fact that some of the seed-borne spores lose their power to germinate during the storage period of grain. The results shown in table 2 are then higher than would be expected under natural farm conditions, and suggest the question as to whether seed treatment of apparently clean seed is a paying proposition for spring plantings.

It was pointed out in the previous paper that seed treatment of such a resistant variety as Marquis is inadvisable when spring seeding is practiced, and it now seems probable that even susceptible varieties may be seeded in the spring without disinfection in case they are clean as far as naked eye examination can reveal, with an assurance that the per cent of smut in the crop will be very low.

This conclusion is borne out by the results shown in table 3. The only samples which carried a sufficient number of spores to be classed as "visibly smutty" were the two Early Baart lots with spore-loads of 6552 and 20,176. The per cents of smut in the "visibly clean" lots

TABLE 3—*Table showing the spore-load and per cent of smut from naturally smutted samples of wheat obtained from various farms. Spring, 1921.*

Variety	No. Samples	No. of Spores per grain	% Smutted plants
Bluestem	6	0, 21, 83, 104, 104, 145	0
"	1	104	0 85
"	1	145	0 9
"	1	166	0 5
"	1	375	0 5
Early Baart	5	0, 41, 42, 124, 229	0
"	1	966	1 0
"	1	6552	20 0
"	1	20,176	19 5
Marquis	6	0, 01, 21, 42, 104, 145	0
"	1	64	0 78
"	1	333	0 8
Hybrid 143	3	125, 125, 187	0
Jenkins Club	1	499	0
Red Chaff	1	270	0

ranged from 0 to 1 with spore-loads from 0 to 966. Since one per cent of smut would have little or no effect upon yield it is doubtful if seed treatment of any of the visibly clean seed would have been a paying proposition.

OVER-SUMMERING OF LEAF RUST OF CEREALS IN KENTUCKY

W. D. VALLEAU

The problem of determining the exact method of over-summering of the leaf and stem rusts of the small grains is an important one in Kentucky. It is comparatively easy to understand how the rusts over-winter once mycelium becomes established in the tissues of plants whose leaves live thru the winter. The small-grain fields go thru the winter often entirely free from either leaf or stem rust, but as conditions become favorable for infections in the spring, wheat fields especially become heavily infected first with the orange leaf rust and later with the black stem rust. This requires that the leaf rust at least must have over-summered the previous season in considerable abundance. The barberry has not been considered a factor in the black stem rust problem in Kentucky but as this disease is very prevalent late in the season on wheat it is possible that it may also over-summer in the same way as the orange leaf rust.

Volunteer wheat (or barley or rye) plants have been considered the host on which the leaf rusts over-winter but little detailed evidence has been published as to when the volunteer plants become established and how the rust is carried over from the infected crop to these plants. It is in an attempt to supply some of these details that this note is written.

In attempting to find suitable material for study of over-wintering of the black stem rust of wheat on the Kentucky Experiment Station farm during the fall of 1922, Mr. J. W. Barringer and the writer found a few plants of volunteer wheat infected with leaf rust in a clover field. As this suggested that volunteer wheat plants in clover fields might be the means of over-summering of both leaf and stem rusts, the writer made further studies of first year clover fields following barley, rye and wheat to determine more accurately the possible relationship between clover or grass fields sown in the small grains and the over-summering of the rusts.

Altho most of the observations have been made the past fall and winter, the history of the rusts from harvest time thru the following summer and winter seems to be so clearly written in the clover fields as to leave little doubt as to the exact method of over-summering.

The barley, rye and wheat in the fields studied was planted in the fall of 1921. In the early spring the fields were sowed to clover. Some

time before harvest, the wheat and barley plants became heavily infected with orange leaf rust. Some lodging was observed during the spring in the barley and wheat fields. At harvest time, the lodged straw and some of the heads were crushed into the ground by the binder thus bringing seeds in close contact with the soil and leaving a mulch of straw over them. At the time the straw and leaves of all three grains bore numerous uredineal pustules of the orange leaf rust. Some of the wheat was also heavily infected with black stem rust. An inspection, in the fall, of the straw of some 50 varieties of wheat preserved in the seed house showed that the uredineal stage of the black stem rust was still present at harvest time. This condition seems usual in Kentucky as the writer has seen wheat fields at harvest time so heavily rusted that men and horses were covered with the brown spores of the black stem rust after running the binder for a short time. Mr. Barringer assured me that this is not the case further north in the winter wheat section. Some of each of the wheat, rye and barley seeds which had been crushed into the ground evidently germinated soon after harvest as evidenced by the fact that in the fall, numerous well established plants surrounded by small masses of dead rusted leaves were found under their respective mulches of straw. Other seeds germinated during the fall. Plants from these when examined late in the fall were characterized by having seedling leaves only and a poorly developed root system. These plants were rarely found to be rusted.

The young plants which developed in the early summer soon after harvest grew up thru the mulch of heavily rusted straw and evidently thus became infected. It is well known that the red rust spores of both the leaf rust and stem rust can survive for a considerable number of days if conditions are not right for germination and thus the primary infection of the volunteer plants may be accounted for. The writer was unable to find volunteer plants either of barley, wheat or rye which had lived thru the summer which were not fairly well covered with straw: while wherever the binder had crushed a mass of plants into the ground, volunteer plants which gave evidence of having over-summered could be readily found. They were always heavily infected with leaf rust. Following infection of the volunteer plants the process of over-summering of the orange leaf rust was very simple. The new leaves as they developed became infected from the older leaves which, in turn, died thus leaving the mass of dead leaves at the base of the plants in the fall. It is a comparatively simple thing the following spring for the leaf rust to spread from these heavily infected volunteer plants in the clover fields into neighboring wheat fields and to cause the severe epidemics which appear each year in this State.

Altho black stem rust of wheat has not yet been found over-summering in this manner by the writer, he sees no reason why it should not do so in view of the fact that uredinia are numerous at harvest time in some fields. If the fungus over-summers in this way, there is little question as to its ability to over-winter as mycelium in the leaves of these volunteer plants and thus account for the rather severe epidemics of black stem rust of wheat which often occur in Kentucky late in the spring.

An examination of rusted wheat, barley, timothy and orchard-grass during the winter has shown that most or possibly all of the leaves bearing rust pustules in the fall die during the winter; but apparently the rusts are capable of living through the winter as mycelium in vigorous green leaves as on March 4, 1923, the writer found a year old timothy plant partly protected by a brick and a light covering of straw which had several apparently newly formed pustules. These, when examined on March 14, appeared old, but a new fresh pustule containing viable spores was found on the same plant. On this date a volunteer barley plant which had lived through the previous summer was found on which there were 32 very small apparently new pustules of leaf rust on green, vigorous leaves. A single small pustule of orange leaf rust of wheat was found on a volunteer wheat plant March 8. All of the pustules found appeared to have resulted from mycelia which had over-wintered in green, vigorous leaves. They were all found in a one-year-old clover field.

As the practice of sowing grasses and clovers in wheat is general thruout much of the winter wheat area in this country, the clover and grass fields with their rusted volunteer wheat plants may be expected to play a prominent part in the over-wintering of the orange leaf rust and they may also prove to be a close rival of the barberry as a cause of epidemics of black stem rust in at least a portion of the winter wheat section.

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FURTHER STUDIES ON POTATO SEED TREATMENT

J. C. GILMAN AND I. E. MELHUS¹

WITH FIVE FIGURES IN THE TEXT

The interpretation of the existing data from experimental plots on potato seed treatment has led to many contradictory statements regarding the value of various seed treatments for controlling seed-borne diseases. This confusion of interpretation of results usually has arisen because an inadequate system of control plots, which, coupled with an insufficient replication of the trials, gave too high an experimental error. In earlier papers the authors^{2,3} pointed out three of the variable factors found in such experiments, viz.: the presence after treatment of a greater or less number of viable pathogenes on the seed piece, the inhibitory rather than fungicidal action of certain chemicals used in seed treatment, and the presence of the pathogene in the soil. A system of check plots which will to a marked degree measure these factors was suggested. This system consisted of the use of clean treated, clean not treated and infected not treated seed; the plots were replicated a sufficient number of times to reduce the experimental error to within the limits of practical certainty.

TRIALS OF 1920, 1921, AND 1922

In 1920 and 1921, further data were gathered on the basis suggested above, which confirmed the previous findings; and, in order to increase the value of the observations, the question of the most accurate "measuring stick" in any set of field experiments was studied, use being made of certain experimental plots on the control of black scurf (*Rhizoctinia solani*) by seed treatment. In 1920 the amount of disease present on the progeny from the different plots was estimated on the basis of count

¹ Published with the approval of the Director of the Iowa Agricultural Experiment Station.

² Melhus, I. E., Gilman, J. C. & Kendrick, J. B.—Fungicidal Action of Formaldehyde. Iowa Agr. Exp. Sta. Res. Bull. 59, 1920.

³ Melhus, I. E., and Gilman, J. C.—Measuring Certain Variable Factors in Potato Seed Treatment Experiments. Phytopath. 11: 6-17. 1921.

and weight of the infected tubers from the plots and the percentage of disease thus computed. In 1921 the amount of disease present in the plots was computed both on the basis of stem lesions; that is, the percentage of plants showing stem lesions due to *Rhizoctonia solani*, and on the basis of the presence of sclerotia on the tubers at maturity. The latter was computed by weight. Both years the plots consisted of 200 hills planted in parallel rows. In 1920 each treatment was replicated 5 and the checks 8 times. In 1921 three series of experiments were made. In the series A, the plots were duplicated, the checks triplicated; in series B, the plots were not repeated, and in series C the plots were in triplicate, the checks being replicated 4 times. In the last series 400 hills were planted and then part of them were dug to study the method of reading results by means of stem lesions. The seed treatment studies, particularly, were hot and cold formaldehyde, and mercuric chloride. Copper sulphate, bordeaux dust, and bordeaux mixture were also considered in one or the other of the two years. The data showing the results of these experiments are brought together in the following tables:

TABLE 1—Results of seed treatment experiments for the control of *Rhizoctonia solani* in 1920.

Row	Treatment	Percentage of tubers showing sclerotia	
		By weight	By count
1	Clean, CH ₂ O 1-240 25° C. 2 hrs.	11.1	10.7
2	Clean not treated	15.2	15.2
3	Infected not treated	24.0	24.7
4	Infected CH ₂ O 1-240 25° C. 2 hrs.	10.5	14.1
5	Infected CH ₂ O 1-120 50° C. 2½ min. Covered 2 hrs.	5.3	7.4
6	Infected CH ₂ O 1-240 6-8° 2 hrs.	17.5	25.1
7	Infected CH ₂ O 1-240 25° 2 hrs.	4.3	5.3
8	Infected HgCl ₂ 1-1000 6-8° 1½ hrs.	11.4	11.0
9	Infected HgCl ₂ 1-1000 25° C. 1½ hrs.	18.5	19.2
10	Infected CuSO ₄ 1-300 25° C. 2 hr.	21.2	20.1
11	Infected CH ₂ O 1-120 50° 2½ min. covered 2 hrs.	16.1	16.7
12	Infected CH ₂ O 1-240 6-8° C. 2 hrs.	23.7	31.8
13	Infected CH ₂ O 1-240 25° 2 hrs.	16.4	19.9
14	Infected HgCl ₂ 1-1000 6-8° C. 1½ hrs.	13.3	12.0
15	Infected HgCl ₂ 1-1000 25° C. 1½ hrs.	18.2	20.3
16	Infected CuSO ₄ 1-300 25° C. 2 hrs.	11.7	12.5
17	Clean CH ₂ O 1-240 25° C. 2 hrs.	16.5	15.9
18	Clean not treated	35.0	33.9
19	Infected not treated	17.8	19.3
20	Infected CH ₂ O 1-240 25° C. 2 hrs.	2.7	3.9
21	Clean CH ₂ O 1-240 25° C. 2 hrs.	14.8	14.3

22	Clean not treated	25 7	27 6
23	Infected not treated	34 1	38 3
24	Infected CH ₂ O 1-240 25° C. 2 hrs.	9 6	8 6
25	Infected CH ₂ O 1-120 50° C. 2½ min. Covered 2 hrs.	12 7	16 8
26	Infected CH ₂ O 1-240 6-8° C. 2 hrs.	33 0	25 0
27	Infected CH ₂ O 1-240 25° C. 2 hrs.	12 4	13 7
28	Infected HgCl ₂ 1-1000 6-8° C. 1½ hrs.	5 0	5 4
29	Infected HgCl ₂ 1-1000 25° C. 1½ hrs.	6 2	7 2
30	Infected CuSO ₄ 1-300 25° C. 2 hrs.	17 6	21 2
31	Clean CH ₂ O 1-240 25° C. 2 hrs.	12 8	15.1
32	Clean not treated	5 5	9.0
33	Infected not treated	47 1	42 5
34	Infected CH ₂ O 1-240 25° C. 2 hrs.	7.6	9 3
35	Infected CH ₂ O 1-120 50° C. 2½ min. Covered 2 hrs.	10.7	12 8
36	Infected CH ₂ O 1-240 6-8° C. 2 hrs.	6 5	8 1
37	Infected CH ₂ O 1-240 25° C. 2 hrs.	12.9	13.7
38	Infected HgCl ₂ 1-1000 6-8° C. 1½ hrs.	19 7	21.5
39	Infected HgCl ₂ 1-1000 25° C. 1½ hrs.	12 5	12 1
40	Infected CuSO ₄ 1-300 25° C. 2 hrs.	23 0	24 2
41	Clean CH ₂ O 1-240 25° C. 2 hrs.	17.6	19 2
42	Clean not treated	11 3	11.9
43	Infected not treated	28 6	24 0
44	Infected CH ₂ O 1-240 25° C. 2 hrs.	14 4	7 9
45	Clean CH ₂ O 1-240 25° C. 2 hrs.	14.8	14.4
46	Clean not treated	21 8	20 5
47	Infected not treated	43.4	39.3
48	Infected CH ₂ O 1-240 25° C. 2 hrs.	21 8	21.0
49	Infected not treated B. D.	17.6	18.0
50	Clean not treated B. D.	46.0	38.3
51	Infected CH ₂ O 1-240 25° C. 2 hrs. B. D.	13.5	13.2
52	Infected CH ₂ O 1-240 25° C. 2 hrs. B. D.	28 3	24.2
53	Infected CH ₂ O 1-120 50° C. 2½ min. B. D. Covered 2 hrs.	36 3	31.1
54	Infected HgCl ₂ 1-1000 25° C. 1½ hrs. B.D.	23.9	20.9
55	Infected CuSO ₄ 1-300 25° C. 2 hrs. B. D.	11.3	12.6
56	Infected CH ₂ O 1-240 25° C. 2 hrs. B. D.	22 8	23.7
57	Infected not treated B. D.	47 3	46.3
58	Clean not treated B. D.	26.8	21.4
59	Clean CH ₂ O 1-240 25° C. 2 hrs. B. D.	20 3	21 0
60	Clean CH ₂ O 1-240 25° C. 2 hrs.	12.7	12.0
61	Clean not treated	46 0	41.3
62	Infected not treated	36.5	33.9
63	Infected CH ₂ O 1-240 25° C. 2 hrs.	32.5	32.6
64	Infected Hot HgCl dip	13.3	13.2
65	Infected Hot CuSO ₄ dip	31.6	34.6
66	Infected Bordeaux paste (10-10-50)	55.5	56.8
67	Infected Bordeaux paste (10-10-50)	38.0	38.8

68	Infected-Paraform dust	37.3	34.7
69	Clean CH ₂ O 1-240 25° C. 2 hrs.	6.8	9.5
70	Clean not treated	25.3	25.0
71	Infected not treated	47.0	55.6
72	Infected CH ₂ O 1-240 25° C. 2 hrs.	13.2	17.0

SUMMARY OF MEANS

Clean treated	12.6±0.79	14.04±0.69
Clean not treated	22.4±2.88	23.92±2.48
Infected not treated	35.4±2.85	34.16±2.48
Infected treated	14.1±2.10	14.32±2.05
CH ₂ O 1-120 50° C. 2½ min.	11.1±1.2	
CH ₂ O 1-240 2 hrs. 25° C.	10.7±1.5	
CH ₂ O 1-240 2 hrs. 6-8° C.	22.3±3.4	
HgCl ₂ 1-1000 1½ hrs. 25° C.	13.9±1.7	
HgCl ₂ 1-1000 1½ hrs. 6-8° C.	12.6±1.6 *	
CuSO ₄ 1-300 2 hrs. 25° C.	18.1±1.8	

B. D. The seed was covered with bordeaux dust just previous to planting.

SUMMARY OF TABLE I

The results of 1920 aside from the data concerning the best means of measuring control, showed that the hot formaldehyde treatment (CH₂O 1-120 50° C. for 2 min.) gave practically as good control as the standard formaldehyde treatment, and a little better control than the standard mercuric chloride treatment. The differences in control are of doubtful significance, while the shorter time for the hot method gives it considerable economic value. An interesting point that should be mentioned is the better control with standard formaldehyde at a warm temperature, 25° C. over cold 6-8° C. In the case of the former, the percentage of disease was cut in half. Copper sulphate did not give any better control and the plots showed injury from this chemical. The four check plots maintained the same relative positions as they have in experiments previously reported¹. Bordeaux paste 10-10-50 and paraform dust were of no control value and very injurious to stand.

In addition to the fungicides mentioned, one set of plots was planted in 1920 in tubers that had been dusted with commercial bordeaux dust just previous to planting and after the tubers were treated and cut. It was thought that the fungicide might act as an antiseptic and prevent the attack of *Rhizoctonia solani*. As will be readily seen from the results, the dusting had little or no effect on the percentage of disease present on the progeny of these tubers, if the percentage of *Rhizoctonia* on the progeny of any plot is compared with the percentage of *Rhizoctonia* on

¹loc. cit.

the nearest plot having the same treatment without the dusting. In 6 out of 11 cases the dusted tubers showed more disease present on their progeny than did those not dusted.

These results were estimated in two ways. The progeny from the plots were sorted into those showing sclerotia and those free from sclerotia, and the two groups were weighed and counted, and the percentage of disease calculated from these figures. In the 72 field plots the percentage by count was greater than the percentage by weight in 35 cases. In 28 cases the percentage by weight was greater than the percentage by count and in 9 cases the percentage of both weight and count were equal. The greatest variation was in plot 26 where the

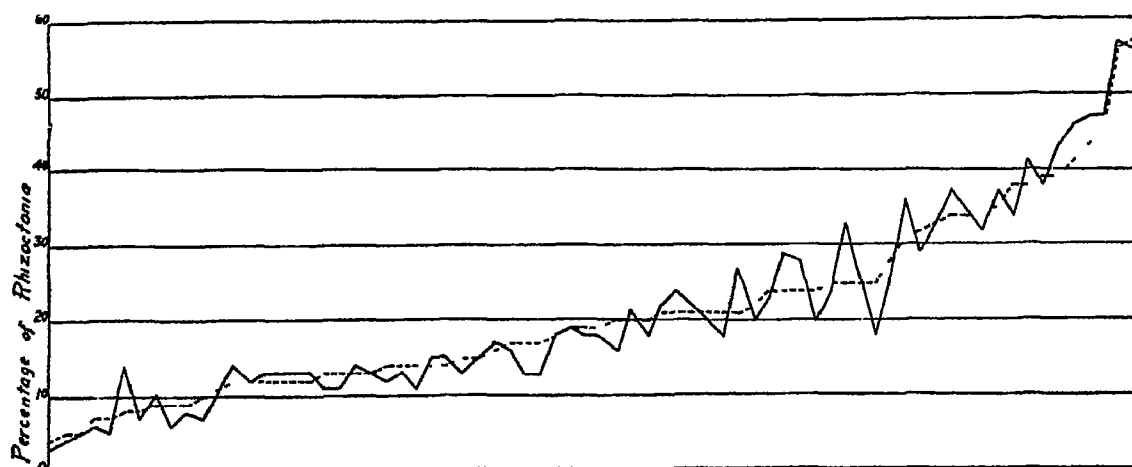


Fig. 1. Comparison of percentage of *Rhizoctonia* estimated by weight with percentage estimated by count.

Percentage of disease estimated by count.....

Percentage of disease estimated by weight_____

percentage by weight was 8 per cent greater than the percentage by count. On the other hand, in the 25 plots showing 25 per cent or more infection the percentage by weight exceeded the count in 16 cases. Figure 1 shows this comparison graphically. An array was made of the percentage values by count and then the percentage value by weight for the same row was plotted. It is apparent that in spite of minor differences the percentage of disease computed by either method is practically the same.

If the mean percentages by count and weight in the four checks which were replicated eight times are compared, we find that in three cases the percentage by count is a little higher than that by weight, but the difference is insignificant. The figures are given in table 1, and shown in histogram form in figure 2. In this figure the solid histograms show the percentage by count; those outlined, percentage by weight.

The means in the checks that were replicated 8 times, show clearly that percentage by weight and count are very close to one another, and therefore it makes little difference whether one uses weight or count as an indicator of degree of control.

It is obvious that the differences are not great and either the count of tubers bearing sclerotia or the weight of the infected progeny may be taken as an indicator of the fungicidal value of a method of seed treatment. From the point of view of mechanical ease and rapidity of performance the measurement by weight is probably preferable.

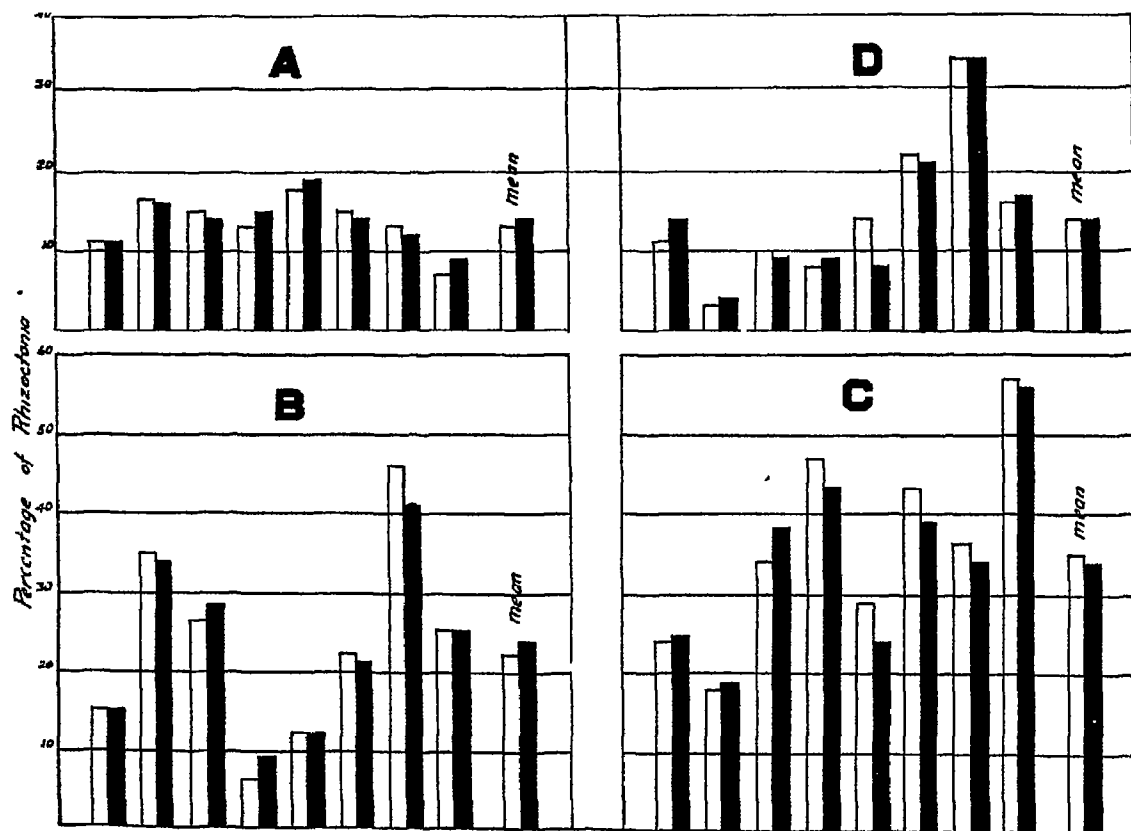


FIG. 2. Comparison of the estimate of *Rhizoctonia* by count and weight on the four check plots. The solid histograms show percentage of disease by count; the outlined, by weight.

A—Clean treated.

B—Clean not treated.

C—Infected not treated.

D—Infected treated.

TABLE 2—Results of seed treatment experiments for the control of *Rhizoctonia solani* 1921.

Row	Treatment	Yield of tubers		
		Total wt. oz.	Infected wt. oz.	Per cent infected
1	Clean treated CH_2O 1-240 25° 2 hrs.	261	67	21.8
2	Clean not treated	378	108	28.5

	3 Infected not treated	284	172	60.5
	4 Infected treated	429	197	45.9
	5 CH ₂ O 1-120 50° C. 2½ min.	736	146	19.8
	6 CH ₂ O 1-240 6-8° C. 2 hrs.	700	250	35.7
	7 CH ₂ O 1-240 25° C. 2 hrs.	514	74	14.4
	8 HgCl 1-1000 6-8° C. 1½ hrs.	721	66	9.1
Series	9 HgCl ₂ 1-1000 25° C. 1½ hrs.	1139	239	20.9
A	10 CaOCl 4 hrs.	678	301	44.3
	11 Clean treated	657	45	6.8
	12 Clean not treated	828	181	21.8
	13 Infected not treated	482	234	48.5
	14 Infected treated	720	224	31.1
	15 CH ₂ O 1-120 50° C. 2½ min.	628	82	13.0
	16 CH ₂ O 1-240 6-8° C. 2 hrs.	789	220	27.8
	17 CH ₂ O 1-240 25° C. 2 hrs.	684	112	16.3
	18 HgCl ₂ 1-1000 6-8° C. 1½ hrs.	742	168	22.6
	19 HgCl ₂ 1-1000 25° C. 1½ hrs.	369	5	1.3
	20 CaOCl 4 hrs.	613	95	15.4
	21 Clean treated	608	37	6.09
	22 Clean not treated	482	36	7.4
	23 Infected not treated	368	86	23.3
	24 Infected treated	665	80	12.0
	25 Infected not treated Bordeaux mixture	500	77	15.4
	26 Clean not treated Bordeaux mixture	669	42	6.2
	27 Clean treated Bordeaux mixture	359	41	11.4
	28 Infected treated Bordeaux mixture	535	28	5.2
	29 CH ₂ O 1-120 50° C. 2½ min. B. M.	679	58	8.5
	30 HgCl ₂ 1-1000 25° C. 1½ hrs. Bordeaux mixture	582	22	3.7
	31 HgCl 1-1000 6-8° C. 1½ hr. B. M.	463	28	6.0
Series	32 CaOCl 4 hrs. B. M.	708	84	11.9
B	33 CH ₂ O 1-240 6-8° B. M.	518	84	16.6
	34 CH ₂ O 1-240 25° C. 2 hrs. B. M.	578	66	11.4
	35 Inf. not treated B. M.	439	93	21.1
	36 Clean not treated B. M.	660	105	15.8
	37 Clean treated B. M.	397	8	2.0
	38 Infected treated B. M.	526	12	2.2
	39 Clean treated	628	19	2.9
	40 Clean not treated	564	28	4.9
	41 Infected not treated	251	0	0
	42 Infected treated	644	56	8.7
	43 Clean treated	434	16	3.6
	44 Clean not treated	434	46	10.5
	45 Infected not treated	262	74	28.2
	46 Infected treated	435	21	4.8
	47 Hot formaldehyde	489	35	7.1
	48 Standard formaldehyde	446	67	15.6

	49 Standard HgCl ₂	339	66	19.4
	50 Clean treated	378	7	1 8
	51 Clean not treated	288	16	5 5
	52 Infected not treated	466	195	41 8
Series	53 Infected treated	457	40	8 7
C	54 Hot formaldehyde	332	55	16 5
	55 Standard formaldehyde	538	75	13 9
	56 Standard HgCl ₂	584	55	9 4
	57 Clean treated	466	20	4 2
	58 Clean not treated	602	33	5.4
	59 Infected not treated	524	266	50.7
	60 Infected treated	576	38	6 5
	61 Hot formaldehyde	420	64	13 2
	62 Standard formaldehyde	517	65	12 5
	63 Standard HgCl ₂	538	46	8.5
	64 Clean treated	466	35	7.5
	65 Clean untreated	330	50	15.4
	66 Infected not treated	391	121	30 9
	67 Infected treated	264	44	16.6

MEANS 1921

Series	Clean treated	9.7 ± 3 0
A	Infected not treated	19 2 ± 3.2
	Infected not treated	43 3 ± 6.1
	Infected treated	27.6 ± 5.5
	CH ₂ O 50° C. 2½ min.	16.7 ± 1.6
	CH ₂ O 6-8° C. 2 hrs.	31.5 ± 1.9
	CH ₂ O 25° C. 2 hrs.	15.5 ± 0.5
	HgCl ₂ 6-8° C. 1½ hrs.	15 9 ± 3.2
	HgCl ₂ 25° C. 1½ hrs.	16.1 ± 5 3
	CaOCl 4hrs.	30.6 ± 6.9
Series	Clean treated	4 5 ± 0.7
B	Clean not treated	6 06 ± 0.6
	Infected not treated	13.8 ± 5.7
	Infected treated	10.4 ± 0 8
	Clean treated and B. M.	6.4 ± 2.2
	Clean not treated B. M.	11.0 ± 2.3
	Infected not treated B. M.	18.1 ± 1.3
	Infected treated and B. M.	3.7 ± 0.7
Series	Clean treated	4.4 ± 0.7
	Clean not treated	8.7 ± 1.4
C	Infected not treated	39 9 ± 3.1
	Infected treated	8.2 ± 1.4
	Hot formaldehyde	12.4 ± 1.5
	Standard formaldehyde	13.7 ± 0.5
	Standard HgCl ₂	11.6 ± 1.9
Summary	Clean treated	6.3 ± 1.4
	Clean not treated	12 7 ± 1.9

Infected not treated	37.9 $\pm$ 4.3
Infected treated	17.9 $\pm$ 3.3
Hot formaldehyde	14.6 $\pm$ 1.3
Standard formaldehyde	14.5 $\pm$ 0.4
Standard HgCl ₂	13.8 $\pm$ 2.3

SUMMARY OF TABLE 2

The results from the plots in 1921 as shown in table 2 confirmed the data of 1920. The percentage of disease was higher in all cases except checks but the relative positions of the treatments, when they are arranged from the standpoint of control is the same. Hot formaldehyde, standard formaldehyde at 25° C., and standard mercuric chloride all gave practically the same control. The difference between standard formaldehyde treatment at 25° C. and 6–8° C. was again marked. The tubers from the latter plots showed twice as much disease as those from the former.

Bordeaux mixture 8–8–50 was substituted for the bordeaux dust used in 1920, the application being made before the tubers were cut. Otherwise the treatment and comparisons are similar. In this case the bordeaux treated plots show less *Rhizoctonia* present on the progeny in 10 out of 14 cases. The beneficial effect was insufficient to warrant the treatment, however. The disease was relatively lessened, but the effect of the first treatment was greater than that of the bordeaux mixture. This is clearly shown in the high percentage of disease present when the infected tubers were treated with the bordeaux mixture alone. The greater fungicidal value of the liquid as compared with the dust is probably due to the action of the copper brought into solution on the sclerotia before planting and not to any protective effect in the ground.

During the course of these experiments, the question arose as to whether the percentage of sclerotia on the progeny was an accurate measure of the effectiveness of a given seed treatment, since the injury caused by *Rhizoctonia* is primarily due to its action on the stem of the growing plant. The deposition of sclerotia on the new tubers might not be a true index of the amount of the fungus that had been present on the seed piece.

TABLE 3—Comparison of stem and tuber infection with *Rhizoctonia solani*, 1921.

Row No.	Treatment	No. Plants	Yields Oz.	Per cent of stem lesions on Rhiz.		Per cent Rhiz. on tubers
				June 3	June 14	
1	Clean treated	48	434	0	9	4.2
8	" "	54	378	0	13	3.6
15	" "	56	466	4		1.8
22	" "	56	466	4		7.5

Mean					3 7 ± 0 9		4 5 ± 0 7
2	Clean not treated	52	434	13 4	20	10 5	
9	" " "	59	288	13 5	26	5 5	
16	" " "	56	602	3 6		5 4	
23	" " "	52	330	3 8		15.4	
Mean					8 6 ± 1 6		8 7 ± 1 4
3	Infected not treated	46	262	43 4	50	28 2	
10	" " "	51	466	50 9	62	41 8	
17	" " "	56	524	47 8		50 7	
24	" " "	54	391	38 8		30 9	
Mean					39 9 ± 3 1		45 4 ± 1 5
4	Infected treated	54	435	11 1	27	16 6	
11	" "	55	457	16 1	18	4 8	
18	" "	59	576	13.5		8 7	
25	" "	54	264	12 9		6 5	
Mean					14 2 ± 0 7		8 2 ± 1 45

SUMMARY OF TABLE 3

In the 1921 trials, Series C, each plot was enlarged to 400 sets so as to allow for the digging of part of the sets after the plants were up. The digging was done when the plants were about 4 to 6 inches tall and the number of plants with and without lesions counted. The results obtained are given in table 3 and shown in figure 3. The shaded histograms represent the percentage of the plants dug that had *Rhizoctonia* lesions; the unshaded columns the percentage by weight of the progeny that showed sclerotia. It is clear that the percentage of plants with lesions and tubers with sclerotia are not always the same, but they have the same general trend and the difference in most cases is but slight. This fact is particularly brought out by the means of the 4 checks in which the differences are less marked than when the individual rows were considered. Stem lesions according to 1921 results, give a similar result to the readings made by means of the deposition of sclerotia on the tubers. The readings obtained by either of these methods probably would not vary materially. It should be stated, however, that the reading on stem lesions should be made early in the life of the plant, for later in the season, as shown in table 3, the fungus spreads from plant to plant and the reliability is thereby lessened, although the relative amount of disease present seems to increase more or less uniformly throughout the plots.

SUMMARY OF TABLE 4

In 1922 the efficiency of hot formaldehyde having been established for Iowa conditions, the efficiency of hot mercuric chloride used alone and in combination with formaldehyde was tested. This combination was made in an attempt to combine the inhibitory action of the mercuric chloride with the fungicidal efficiency of the formaldehyde. The plan of the experiments differed from that used in the preceding trials, in that 8 replications of each plot and 10 replications of each of the 4 check plots were used. This change was made due to the fact that but 100

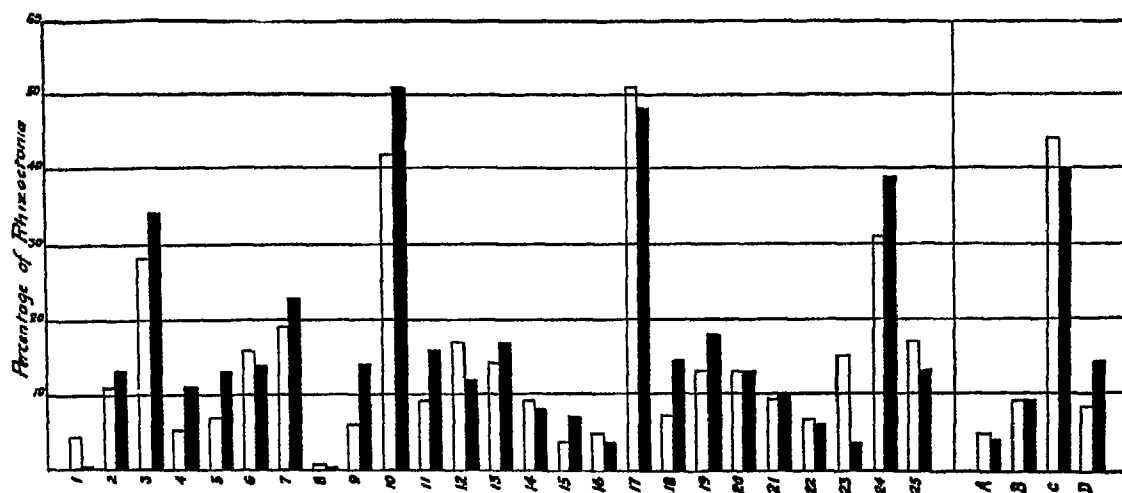


FIG. 3. Comparison of the estimate of the percentage of Rhizoctonia by means of stem lesions (shaded), and the estimate by weight of diseased progeny (outlined).

1, 8, 15, 22—Clean treated.

2, 9, 16, 23—Clean not treated.

3, 10, 17, 24—Infected not treated.

4, 11, 18, 25—Infected treated.

5, 12, 19—Hot formaldehyde.

6, 13, 20—Standard formaldehyde.

7, 14, 21—Standard mercuric chloride.

A—mean of clean treated.

B—mean of clean not treated.

C—mean of infected not treated.

D—mean of infected treated.

sets could be placed in each row. The plots were so arranged, however, that each treatment occupied two adjacent rows which could be combined to constitute a plot comparable to those used in the previous years. In addition since laboratory tests have shown a slight difference between the fungicidal efficiency of mercuric chloride made up in distilled water and that made up in tap (hard) water, plots from potatoes treated in these two solutions were added. The results of this year's experiments are given in table 4.

TABLE 4—Results of seed treatment experiments for the control of *Rhizoctonia solani*, 1922.

Row	Treatment	Yield of tubers		Per cent infected
		Total wt. oz.	Infected wt. oz.	
1	Clean treated	232	8	3.4
2	" "	356	56	15.7
3	Clean not treated	344	52	15.1
4	" " "	—*	—	—
5	Infected not treated	—*	—	—
6	" " "	186	84	45.1
7	Infected treated	508	106	20.8
8	" "	480	86	17.9
9	HgCl ₂ 1-1000 2 min. 50°	600	468	78.0
10	" " " "	355	199	56.0
11	HgCl ₂ 1-2000 plus CH ₂ O 1-240 2 min. 50°	468	304	64.9
12	" " " " " " "	657	236	35.8
13	CH ₂ O 1-240 2 hrs.	584	168	28.9
14	" " "	446	154	34.5
15	Tap H ₂ O HgCl ₂ 1-1000	586	98	16.7
16	" " " "	922	318	34.4
17	Dist. H ₂ O HgCl ₂ 1-1000	600	236	39.3
18	" " " "	372	148	39.8
19	Clean treated	609	160	26.2
20	" "	567	176	30.9
21	Clean not treated	720	104	14.4
22	" " "	628	178	28.3
23	Infected not treated	818	162	19.8
24	" " "	462	166	34.9
25	Infected treated	542	184	33.9
26	" "	644	204	31.6
27	HgCl ₂ 1-1000 2 min. 50°	612	216	34.2
28	" " " "	594	272	45.9
29	HgCl ₂ 1-2000 & CH ₂ O 1-240 2 min. 50° C.	750	142	18.9
30	" " " " " " "	450	82	18.2
31	CH ₂ O 1-240 2 hrs.	448	94	20.9
32	" " "	340	50	14.7
33	Tap H ₂ O HgCl ₂ 1-1000	312	58	18.5
34	" " " "	350	22	6.2
35	Dist. H ₂ O HgCl ₂ 1-1000	562	74	13.1
36	" " " "	492	62	12.6
37	Clean treated	604	18	2.9
38	" "	304	58	19.0
39	Clean not treated	610	105	17.2
40	" " "	420	66	13.3
41	Infected not treated	520	200	38.4
42	" " "	368	128	34.8
43	Infected treated	624	82	13.1
44	" "	631	104	16.4
45	HgCl ₂ 1-1000 2 min. 50°	506	82	16.2

46	HgCl ₂ 1-1000 2 min. 50°	315	82	26 0
47	HgCl ₂ 1-2000 ± CH ₂ O 1-240 2 min. 50°	418	44	10 5
48	" " " " " " "	382	114	29 8
49	CH ₂ O 1-240 2 hrs.	534	82	15 3
50	" " "	432	104	24 0
51	Tap H ₂ O HgCl ₂ 1-1000	470	100	21 2
52	" " " "	534	114	21 3
53	Dist. H ₂ O HgCl 1-1000	662	82	12 3
54	" " " "	560	128	22 8
55	Clean treated	584	40	6 8
56	" "	616	122	19 8
57	Clean not treated	666	118	17.7
58	" " "	434	92	21.1
59	Infected not treated	428	120	28.0
60	" " "	400	162	40 5
61	Infected treated	430	60	13.0
62	" "	470	66	14 0
63	HgCl ₂ 1-1000 2 min. 50° C.	462	142	30 6
64	" " " "	476	71	14.9
65	HgCl ₂ 1-2000 ± CH ₂ O 1-240 2 min. 50° C.	644	172	26.7
66	" " " " " " "	542	148	27.3
67	CH ₂ O 1-240 2 hrs.	430	78	18.1
68	" " "	552	100	18.1
69	Tap H ₂ O HgCl ₂ 1-1000	587	20	3.4
70	" " " "	496	120	24.0
71	Dist. H ₂ O HgCl -1000	542	70	12.9
72	" " " "	582	110	18.8
73	Clean treated	596	72	12 0
74	" "	546	38	6.9
75	Clean not treated	526	92	17.5
76	" " "	600	92	15.3
77	Infected not treated	604	288	47.6
78	" " "	509	164	32 2
79	Infected treated	440	30	6.7
80	" "	430	32	7.4

Summary

Clean treated	5015	750	14.9 ± 2.3
Clean not treated	4948	899	18.1 ± 0.9
Infected not treated	4295	1474	34.3 ± 1.8
Infected treated	5299	954	18.0 ± 1.9
HgCl ₂ 1-1000 2 min. 50° C.	3920	1532	39.0 ± 4.8
HgCl ₂ 1-2000 ± CH ₂ O 1-240 2 min. 50° C.	4212	1242	29.4 ± 5.0
CH ₂ O 1-240 2 hrs.	3766	830	22.0 ± 1.5
Tap H ₂ O HgCl ₂ 1-1000	4257	850	19.9 ± 2.2
Dist. H ₂ O HgCl ₂ 1-1000	4472	910	20.3 ± 2.6

* Rows 5 and 6 were lost at harvest time.

In these plots the amount of diseased progeny in the four check plots remained in approximately the same relation to each other as they held in the three preceding years. The infected treated checks showed approximately the same control as the clean not treated checks. In 1921 the latter were better than the former, while in 1920 the reverse was the case. These facts would indicate that selection of sclerotia-free tubers was approximately as good a control as is seed treatment. The difficulty of securing clean seed, however, makes treatment necessary.

Treatments with mercuric chloride 1-1000 at 50° C. for 2 min., and mercuric chloride 1-1000 plus formaldehyde 1-240 for 2 min. at 50° C. gave no better control than did the untreated checks. In fact, these treatments were of no value for the control of the disease during this season.

Although laboratory experiments as published earlier¹ showed greater fungicidal action of mercuric chloride 1-1000 made up with distilled water, over the same solution made up with tap water (hard water), the field experiments show this difference to be of little practical significance. The tap water solution gave 19.9 ± 2.2 per cent of the progeny diseased, while the distilled water solution gave 20.3 ± 2.6 per cent diseased progeny.

DISCUSSION

The literature shows many different statements as to the relative efficiency of different methods of seed treatment. In 1919, 1920 and 1921 a comparison has been made between the standard cold mercuric chloride and formaldehyde methods and the hot formaldehyde method. Each season each plot consisted of 200 sets replicated from 4 to 6 times. The seed used in these trials each year was as uniformly infected with *Rhizoctonia* as it was possible to obtain it. The seed in every case was northern seed having been grown either in South Dakota or Minnesota. The percentage of tubers infected with *Rhizoctonia* sclerotia at harvest time has been used as a "measuring stick."

It is interesting to note the variation in the different plots receiving the same treatment the same year. Note especially the spread in the different plots treated with the hot formaldehyde method in 1919 as shown in figure 4.² It varied from 8 to 20 per cent, cold formaldehyde

¹ Gilman, J. C. Effect of hardness of water on the fungicidal value of mercuric chloride solutions (By title) *Science* n. s. 56: 262. 1922.

² The data for 1919 from which this figure was drawn is given in table IX, Melhus, I. E. and Gilman, J. C. Fungicidal action of formaldehyde. *Iowa Agr. Exp. Sta. Res. Bull.* 59: 378. 1920.

from 14 to 21, and, mercuric chloride from 6 to 21 per cent. The means in the order mentioned are 15 ± 1.6 , 17 ± 1.2 , and 14 ± 2.3 respectively, an extreme difference of only 4 per cent. This percentage may well be within the limit of the experimental error. The subsequent two years show similar fluctuations with means differing even less than 4 per cent as in 1919.

In 1920 the mercuric chloride plots showed a variation of 6 to 18 per cent, with a mean of 13.9 ± 1.7 ; the hot formaldehyde plots, a range of 5 to 16 per cent with a mean of 11.1 ± 1.2 , while standard

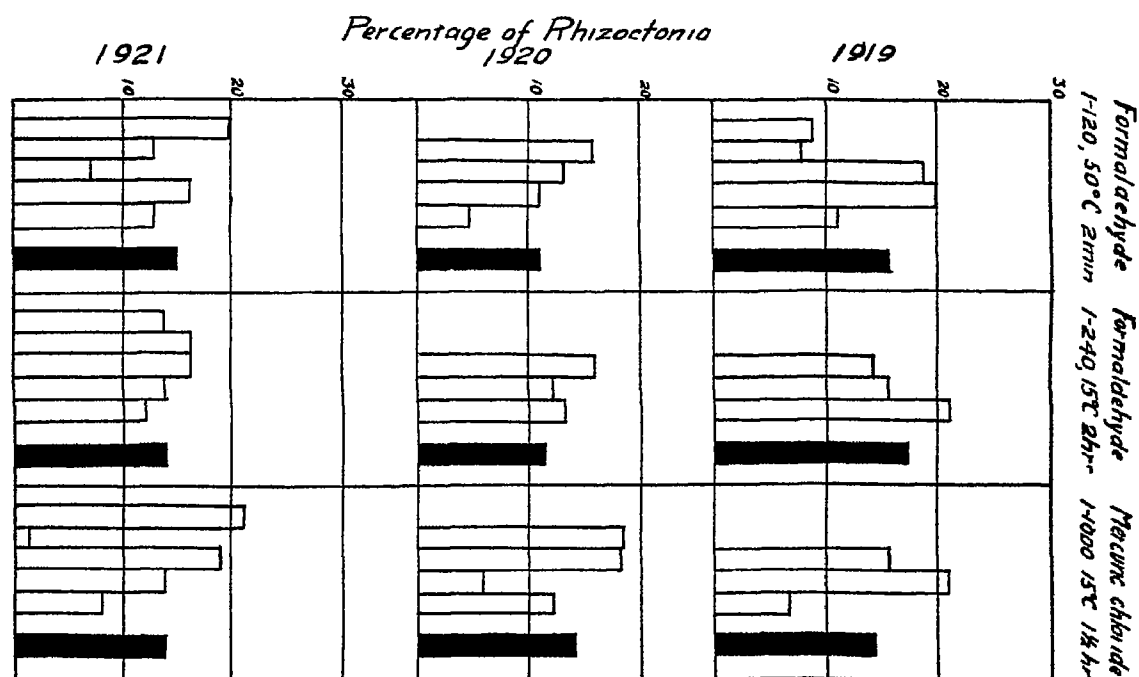


FIG. 4. Comparison of the percentage of *Rhizoetonia* on progeny from seed treated with hot formaldehyde, standard formaldehyde, and standard mercuric chloride for the years 1919, 1920, and 1921. The shaded histograms show the mean percentage of disease for each series of plots.

formaldehyde showed a range of 4 to 16 per cent with a mean of 10.7 ± 1.5 . In 1921 the range for the mercuric chloride plots was 1 to 21 per cent with a mean of 13.8 ± 2.3 ; that for hot formaldehyde was from 7 to 20 per cent with a mean of 14.6 ± 1.3 , and standard formaldehyde varied from 12 to 16 per cent with a mean of 14.5 ± 0.4 . The mercuric chloride plots showed the greatest variation about the mean while the formaldehyde plots were more uniform throughout the three years experiments.

The mean of the three years for the three different treatments is practically the same or about 14 per cent. These results are graphically represented in figure 4. They should be compared with the summary of

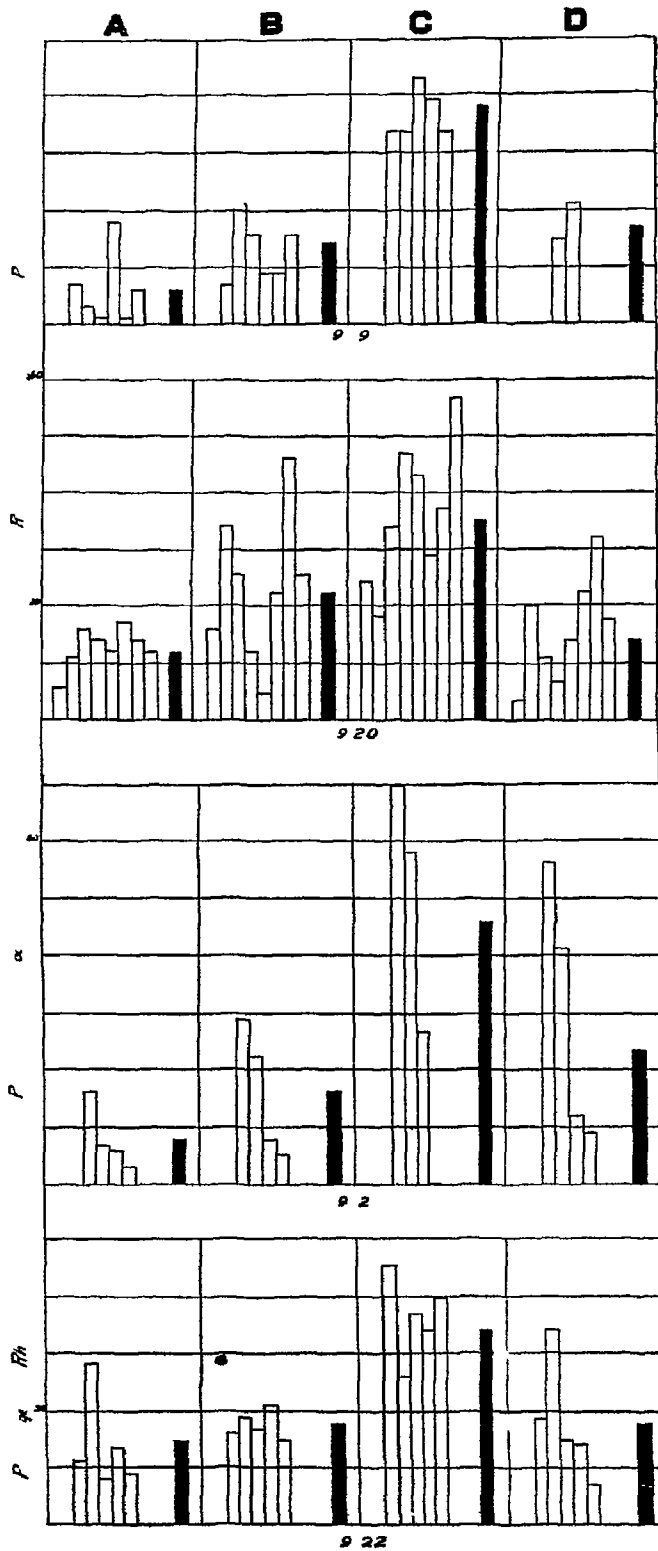


FIG 5 Comparison of the percentage of Rhizoctonia in the four different check plots over the four years experiments The shaded histograms are the means for each series

A—Clean treated

B—Clean not treated

C—Infected not treated

D—Infected treated

the check plots figure 5, for the same years, and it will be readily seen that the variability is in most cases less for the different plots than for the check plots. It is this great variability of results which necessitates the replication in order to reach a fair conclusion as to the efficiency of a given treatment.

In the four years' experiments here considered the check plots were replicated 6 times in 1919, 8 times in 1920, 4 times in 1921, and 5 times in 1922. From the data gathered the relative position of the means remains the same, as regards one another, even when but 4 replications were used and in spite of the fact that in 1920 one of the infected not treated plots gave only a trace of *Rhizoctonia sclerotia* on its progeny. The differing weather conditions, of course, make it impossible to compare the amount of disease except the relative infection resulting in the series of years with the same treatments. From these results four replications seem to be sufficient in the case of the plots used here. Plots of different size or shape might of course require more or might do with less replications but with 200 hills in each plot planted in a row, the plots being parallel, four times seems adequate repetition in ordinary experimental fields. Plots with a fewer number of variable factors such as variety tests or yield, may show significant differences with a less number of replications.

SUMMARY

In potato seed treatment experiments with *Rhizoctonia solani* the efficiency of the treatment can be measured with approximately the same accuracy either by weight or count of the percentage of diseased progeny resulting from that treatment.

Percentage of plants showing stem lesions and percentage of diseased progeny also were of relatively equal value as methods of measuring the efficiency of the fungicides used in seed treatment experiments if the stem lesions were read early in the growing season.

Results obtained over a period of three years showed that hot formaldehyde, cold formaldehyde and mercuric chloride were practically equally efficient from the fungicidal effect produced in controlling *Rhizoctonia solani*. The mean percentages of infected tubers for 1919 were 15 ± 1.6 , 17 ± 1.2 , and 14 ± 2.3 per cent respectively. For 1920 they were 11.1 ± 1.2 , and 10.7 ± 1.5 and 13.9 ± 1.7 . For 1921 they were 14.6 ± 1.3 , 14.5 ± 0.4 and 13.8 ± 2.3 . The results here given show that the formaldehyde experiments gave more constant results than mercuric chloride as evidenced by the greater probable error in the latter plots.

Bordeaux mixture applied as a dust to potato tubers had little fungicidal value as a protection against *Rhizoctonia solani*. Bordeaux mixture 8-8-50 applied as a liquid in which the potatoes were dipped showed a slight fungicidal effect, but one insufficient to warrant its adoption as a practice.

From four years study of seed treatment plots when the plots are 200 hills long in parallel rows, 4 replications will insure fairly reliable results on ordinary experimental fields.

Hot mercuric chloride and a combination of mercuric chloride and formaldehyde showed practically no control of *Rhizoctonia* in 1922.

BOTANY AND PLANT PATHOLOGY SECTION,

IOWA AGRICULTURAL EXPERIMENT STATION.

THE BLACKLEG DISEASE OF THE POTATO. ON THE RELATIONSHIP OF *BACILLUS ATROSEPTICUS* AND *BACILLUS SOLANISAPRUS*

SYDNEY G. PAINE AND H. CHAUDHURI

The two organisms *Bacillus atrosepticus* van Hall. and *Bacillus solanisaprus* Harrison produce diseases of potato tubers and stems which, though very similar, bear certain distinguishing features, namely, the effect of the former in producing a melanin reaction in both the tuber and stem which does not appear as an effect of the latter, and the hardening of the vascular bundles by the former but not by the latter; this hardening of the vascular structure causes the "blacklegged" stems to stand erect in the field whereas in the disease described by Harrison (1) caused by *Bacillus solanisaprus* the rotted stems show a dark green, not a black, colour and are so soft that they fall to the ground. Besides these differences between the symptoms which each respectively produces the two organisms exhibit certain cultural differences, notably, in their colony formation, in their power to liquefy gelatine and in gas formation from sugars; *B. atrosepticus* rapidly liquefies gelatine while *B. solanisaprus* is virtually a non-liquefier.

In spite of these differences, however, the experiments of W. J. Morse (2) seemed to show them to be strains of one and the same species; in these experiments, consisting of prolonged cultivation through several transfers in the same medium, the distinguishing characters disappeared and both gave identical reactions. In an earlier communication E. F. Smith (3), describing *Bacillus phytophthorus* Appel, gives his opinion that "*Bacillus solanisaprus* is a closely related, though not identical, organism and the same may be said of *Bacillus atrosepticus*."

Now these organisms have been in the collection of type cultures in the Imperial College laboratory since 1917 during which period they have been "carried on" side by side on the same media and yet have always been quite easily distinguished by their manner of growth. It was therefore considered advisable to repeat the work of Morse and to extend it in other directions in order to settle this question of the relationship of the two species.

The cultures under examination were one of *B. atrosepticus*, in all probability identical with *B. phytophthorus*, isolated by one of us (S.G.P.) in 1917 and one of *B. solanisaprus* obtained in that year from the American Museum of Natural History. Following closely the plan of the experiments of Morse the cultural and physiological characters of these two organisms were compared before and after a series of daily

transfers from tube to tube of nutrient broth. Of the results of these experiments it suffices to say that we were unable to confirm the finding of Morse. With our cultures and in our hands the distinguishing features of the two organisms described above were present equally at the end of this period of attempted "training" as at the beginning of it.

DETERMINATION OF THE THERMAL DEATH POINT

Capillary tubes filled with suspensions of the organisms from an agar slope of 24 hours' growth at 20° C. were heated for ten minutes and the contents plated out and incubated with the following results:—

Temperature	<i>B. atrosepticus</i>	<i>B. solanisaprus</i>
45° C.	20 Colonies	∞ Colonies
47° C.	Nil	∞ Colonies
50° C.	Nil	110 Colonies
54° C.	Nil	3 Colonies

∞ = innumerable.

B. solanisaprus is here seen to be considerably more resistant to heat than *B. atrosepticus*.

INOCULATION EXPERIMENTS

(a) *Inoculation of Potato Stems.* Allowing the organisms to enter the cut end of a stem immersed in a bacterial suspension in water, or prick inoculation of the growing stem gave always consistent differences, namely, a strong melanin reaction and fishy smell with *B. atrosepticus* and a dark green, water-soaked stem and absence of fishy smell with *B. solanisaprus*.

(b) *Surface Inoculation of Sterile Potato Slices.* Both organisms produced very active rotting; *B. atrosepticus* gave its characteristic pink colour and black (melanin) margin to the rotted area accompanied by the fishy smell of trimethylamine, whereas the rot produced by *B. solanisaprus* was a white rot almost devoid of smell. Moreover, the rots in the two cases were of an entirely different chemical order, as shown by the change in hydrogen ion concentration of the rotted tissue. The original Ph. of the potato juice was 6.4 and the final value for the tissue rotted by *B. atrosepticus* was 7.2 as against 4.8 by *B. solanisaprus*.

(3) *Surface Inoculation of Potato Slices at Different Temperatures.* The diameter of the rotted area resulting from equal loop surface inoculations was measured in millimetres after 20 hours' incubation at the temperatures given below:—

	3°	12°	25°	30°	32°
<i>B. atrosepticus</i>	nil	slight rot 20 mm. diam.	active 45 mm. diam.	active 55 mm. diam.	very active 60 mm. diam. slice completely rotted
<i>B. solanisaprus</i>	slight rot 15 mm. diam.	active 50 mm. diam.	complete 60 mm. diam.	complete 60 mm. diam.	complete 60 mm. diam.

The three sets of experiments clearly establish the two organisms as distinct. The authors have been unable to confirm the work of Morse. In searching for an explanation of his results it is conceivable that his cultures were mixed ones obtained from diseased potatoes bearing both species and that by his cultural treatment the one was specially favoured and the other completely eliminated.

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON.

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A NEW LEAF SPOT DISEASE OF ONION AND GARLIC

COLIN B. WELLES

WITH PLATE XVII AND ONE FIGURE IN THE TEXT

A severe leaf spot of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) has been observed since January 17, 1922, at the College of Agriculture, Los Banos, P. I. The disease was first noticed on garlic which on January 31 was so seriously parasitized that it was doubtful if any of the plants would develop to maturity. In the present paper a description is given of the disease and of the causal organism. The writer is publishing these results as a provisional record until the group to which this organism belongs may be satisfactorily classified.

SYMPTOMS

The disease has been found on onion and garlic and on each host the symptoms appear slightly different (Plate XVII A).

On onion: Frequently all of the leaves of a plant are diseased, showing various gradations in seriousness from slight chlorosis to death. Young lesions appear as circular yellow, chlorotic spots, 5 to 3 mm. in diameter. They are most numerous at the outer end of the leaves and gradually decrease in number towards the base. In slightly older attacks the chlorotic spots coalesce at the end of the leaf. Below there are numerous separate lesions giving a mottled appearance.

Later on, the end dries out and turns grayish brown and less frequently the single lesions develop a brownish center made up of dead tissue. In most severe attacks the entire leaf is killed and turns grayish brown.

Fruiting bodies are visible on the older, dead portions of the lesions, or they may appear as black specks on the chlorotic tissue.

On garlic: The distribution and appearance of lesions on garlic leaves are strikingly different from those on the onion. In the first place garlic has flat leaves and the lesions penetrate through the leaves when they are very young. The lesions commence as small, round or irregular chlorotic spots, spreading rapidly until the entire outer end of the leaf is killed. Instead of the appearance of more small lesions below the margin of this lesion the fungus develops and seems to proceed in a mass down the leaf. The diseased tissue is delimited by a narrow band of chlorotic tissue about 3 mm. in width.

After the original lesions fuse, progress of the disease is rapid and it is only a short time until the leaf is killed. Since most leaves show lesions, death of the plants commonly results from infection.

HOSTS PARASITIZED

Inoculations made recently by the writer have shown that *Cercospora* are very indiscriminate with regard to hosts attacked. It has been found that a species of *Cercospora* isolated from *Phaseolus lunatus* parasitized all legumes on which it was placed and also parasitized many non-leguminous plants. In view of such varied parasitism, it is impossible to state whether onion and garlic are the only hosts of this organism. However, the organism found on garlic and the one found on onion appear to be the same according to present methods of classification.

ECONOMIC IMPORTANCE

Garlic appears to suffer more from the disease than onion, due, perhaps, to the fact that the garlic plants were more mature and had been exposed to greater possibilities of severe infection.

Out of 198 onion seedlings observed on January 20, four had been killed outright, fifty-four were very seriously diseased, while the remaining 138 had from one to five lesions on each leaf.

A second count of the onion plants on February 6 showed that out of a total of 208 plants observed, 17 were healthy, 117 were slightly diseased, 71 severely diseased, and 3 killed. This shows that the disease was becoming more serious, at least in number of plants attacked. On February 17, from a third count of a total of 186 onion plants, it was found that 2 were healthy, 111 were slightly diseased, 69 were seriously diseased, and 4 were dead. This count, while it presents the actual conditions of the plants on the above date, does not make allowance for dead and fallen leaves which appeared abundantly over the entire patch or for the loss of 22 plants which disappeared after the second count. In each instance the same plants were observed.

On the other hand, garlic suffered much more severely. Out of 230 plants recorded 30 were healthy or so slightly diseased that lesions were not noticeable, 32 plants were slightly diseased, 113 plants were seriously diseased, and 55 killed outright. This count was made on January 17.

On February 2, out of a total of 127 garlic plants, none were healthy, 22 were slightly diseased, 54 were seriously diseased, and 51 entirely dead. From this it appears that 103 out of 230 plants disappeared between January 17 and February 2 or a loss of 44.7 per cent of the plants.

The loss continued even after the plants had been sprayed with Bordeaux mixture on January 24.

Plate XVII B shows the relative size of bulbs obtained from diseased and from healthy plants.

CAUSAL ORGANISM

The organism responsible for the leaf spot disease of onion and garlic belongs to the genus *Cercospora* and so far as can be ascertained is an undescribed species.

The conidiophores arise in stomata or from injured surfaces of the leaves. They are produced in bunches and in every detail are similar to many of the common *Cercospora*. The conidiophores are light brown, and measure 47.4 to 165.9 by 5.1 to 7.9 micra with two or three septa when taken from onion, and 50.5 to 168.0 by 5.5 to 8.6 micra with three or four septa when taken from garlic.

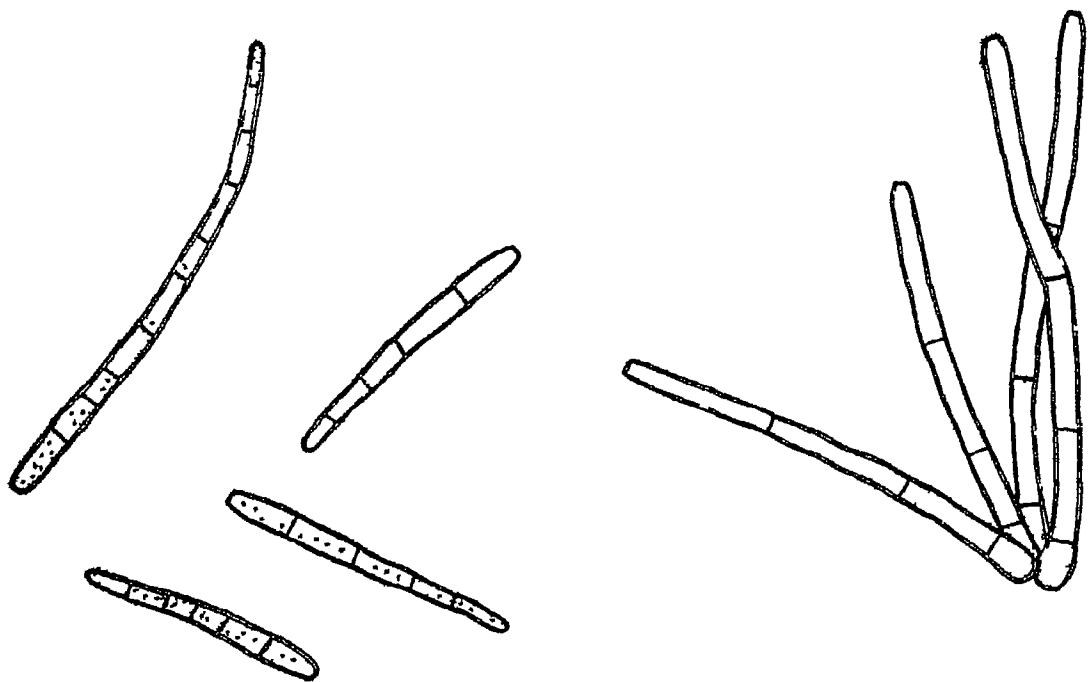


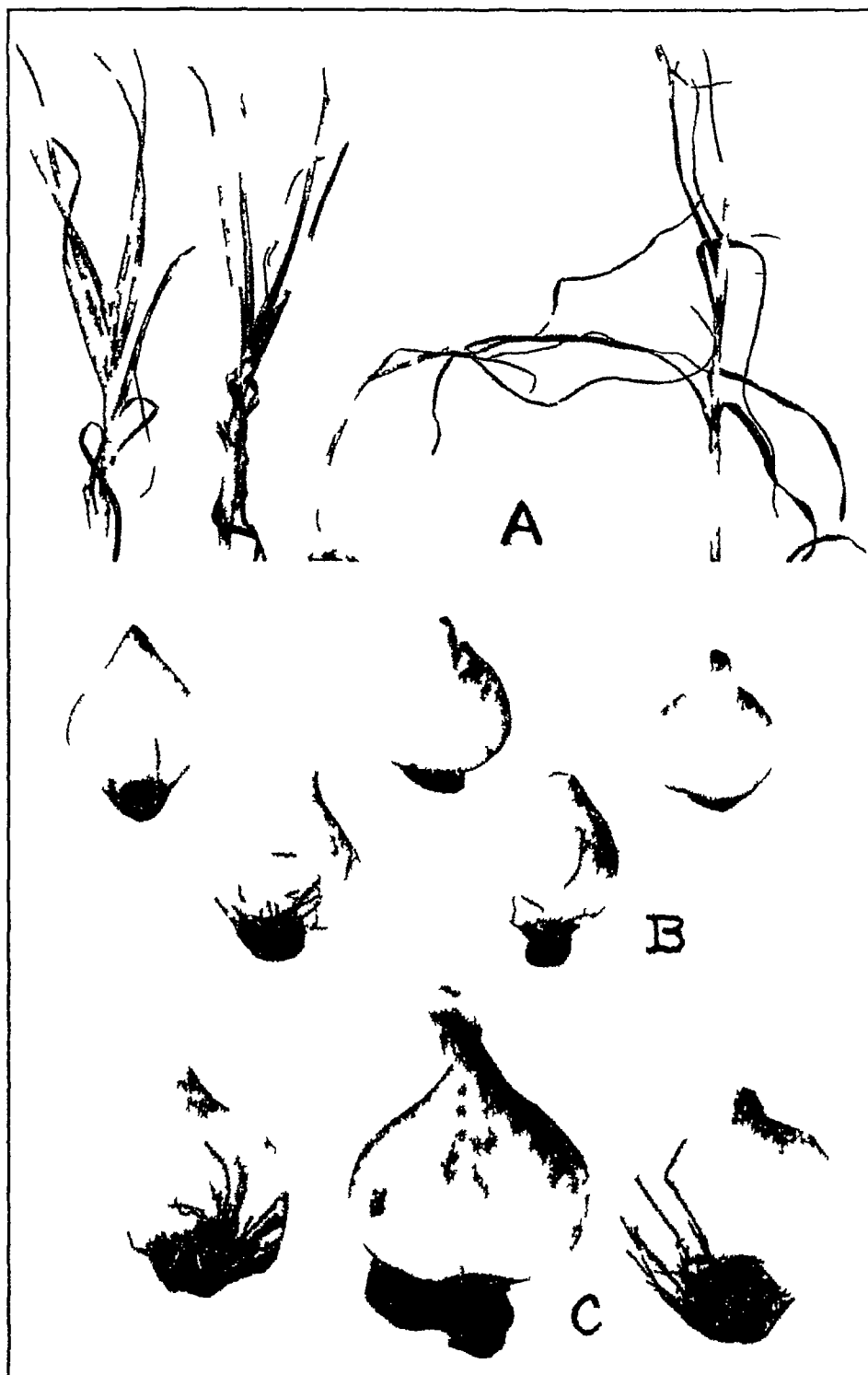
FIG. 1. Conidiophores and conidia of *Cercospora duddiae* n. sp., taken from *Allium cepa*. Drawn with the aid of camera lucida. $\times 900$.

For the most part the conidia are borne terminally on the conidiophores are straight or slightly curved, are moderately short, are thick at the basal end, tapering to a blunt point, and are hyaline. They measure from 48.4 to 79.0 by 6.3 to 7.9 μ with from 3 to 12 septa when taken from onions, and 71.1 to 99.0 by 5.3 to 6.4 μ with from 2 to 15 septa when taken from garlic (Fig. 1).

The name *Cercospora duddiae* is suggested for this organism.

***Cercospora Duddiae* n. sp.**

Spots amphigenous, at first round, later irregular with a border of yellow, chlorotic tissue, having grayish brown centers, 3 to 5 mm. in diameter; conidiophores fasciculate, filiform, simple, frequently genic-



CERCOSPORA DUDDIAT ON ALLIUM SATIVUM

A Plants of garlic *Allium sativum* showing lesions caused by *Cercospora allii*. The plants to the left are slightly diseased those to the right are nearly dead.

B Bulbs from healthy garlic plants. C Bulbs from diseased garlic plants. B and C same scale.

ulate, 47.4 to 168.0 by 5.1 to 8.6 μ , 2 to 4 septate, light brown; conidia slender, straight or slightly curved tapering from basal end to blunt point, 48.4 to 99.0 by 5.3 to 7.9 μ , 3 to 15 septate, hyaline.

On leaves of onion, (*Allium cepa* L.) and garlic (*Allium sativum* L.) found at Los Banos, Laguna, Philippine Islands.

CONTROLS

The disease on garlic was first observed on January 17 and on January 23 the entire plot of plants was sprayed with Bordeaux mixture. The disease was very serious at that time and little if any benefit resulted from the application.

In view of the fact that the disease is confined to the leaves, the application of Bordeaux mixture as a protective measure should give an efficient control. As the disease has appeared during dry weather, a change in horticultural practice would be of little if any benefit, as *Cercospora* appear to thrive most luxuriently during periods of heavy precipitation.

SUMMARY

1. A leaf spot of onion (*Allium cepa* L.) and of garlic (*Allium sativum* L.) has been found at Los Banos, P. I. The disease was so serious that the respective crops were reduced approximately fifty per cent.

2. The fungus has been found on onion and garlic although it may be rather omniverous as other *Cercospora* have been shown to be by inoculation experiments.

3. The causal organism is a *Cercospora* believed to be an undescribed species.

4. The name *Cercospora duddiae* n. sp. is suggested to designate the fungus.

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NOTES ON THE OCCURRENCE AND GROWTH OF CANKERS OF *ENDOTHIA PARASITICA*

N. REX HUNT

During the years 1913-15, the writer conducted a series of spraying tests with lime-sulphur and with Bordeaux mixture as a possible means of controlling chestnut blight. The experimental orchard was located on a shelf near the summit of the Blue Ridge Mountains, fifty miles west of Washington. This shelf slopes gradually down and to the east and is bounded on the west by a short, sharp incline at the top of which is the summit of the ridge and the Virginia-West Virginia state line. The orchard was composed of grafted coppice and native trees mixed. A very few of the grafted trees were Japanese chestnuts, a few were of a superior stock of native chestnuts and the larger part were of the Paragon and related varieties of European chestnuts.

Groups of infected trees were found in the woods to the south and the west of the orchard. Spraying was done with a high pressure power outfit.

A study of the rate of growth of cankers was made (Table 1) and this was used to calculate the probable dates of infection of cankers found (Table 2). The location of cankers was noted (Tables 3, 4) as a possible clue to the source of the spores and to the means of infection. Notwithstanding the enormous number of spores produced by the organism, it seems evident that a relatively low percentage of wounds became infected. Every tree was wounded in nailing on serially numbered brass discs. Countless thousands of wounds were made in cutting and dragging the heavy growth of bush out of the orchard. During the winter of 1914-15 two severe sleet storms broke thousands of twigs and branches. Proximity to diseased trees did not insure quick infection of a tree (Table 5). The amount of growth in large cankers is compared to growth in small cankers and the amount of growth in cankers on grafted trees is compared to growth in ungrafted trees (Table 6). Grafted trees did not blight as readily as ungrafted trees (Table 7). Sprayed trees did not show as much blight as unsprayed trees (Table 8), but spraying was relatively ineffective and not of sufficient benefit to justify the cost.

TABLE 1—*Growth of chestnut blight cankers*

Period of Growth	Winter Sept. 23, 1913 to May 6, 1914	Summer May 6, 1914 to Oct. 23, 1914.	Winter Oct. 23, 1914 to May 14, 1915.	Summer May 14, 1915 to Aug. 26— Sept. 9, 1915.	Fall Aug. 26— Sept. 9 to Nov. 1, 1915.	Fall 1914 to Fall 1915.
No. cankers	3	17	44	30	72	26
Increased diameter of cankers—vertical	1.58 in.	6.06	2 3	4.	1.77	7.8
Monthly rate— “	.22	1.01	.33	1 14	.88	.65
Increased diameter of cankers—horizontal	5	4.82	1 8	2.85	1.14	5.8
Monthly rate— “	.07	.8	.26	.81	.57	.48

When a new canker was found, it was measured and its probable date of infection calculated on the assumption that it had grown at the average rate during each preceding period of time for which this rate had been determined. For example, if we found a canker with a nine inch vertical diameter on November 1, 1915, we would subtract 8.07 inches, the total average growth ($1.77 + 4.0 + 2.3$) from October 23, 1914 to November 1, 1915, and assume that the canker had a vertical diameter of 0.93 inches on October 23, 1914. The average rate of growth during the period ending on October 23, 1914 was 1.01 inches per month. $\frac{.93}{1.01}$ of 30 days = 28 days. Twenty-eight days previous to October 23 would be September 25, 1914. If the similar calculation based on the horizontal diameter threw the infection date back to September 15, the calculated date of infection would be made half way between or September 20, 1914. Many of the cankers were rather small when found and the margin of error was correspondingly smaller.

Winter sleet injuries and increased supplies of spores help to account for the large number of early infections in 1915. The late season infections would not become sufficiently conspicuous to be found during that year.

Numbers in parentheses indicate cankers appearing where tags were nailed on trees. Nearly all of these tags were nailed on the east or on the west sides of the trees. Even without the cankers resulting from

TABLE 2—*Probable infection dates as calculated from the data in table 1.*

	1913	1914	1915
April		1	21
May		6	9
June		5	18
July		10	8
August	1	9	10
September		16	2
October	1	14	1

TABLE 3—*Location of chestnut blight cankers on trees.*

Side of tree on which basal or body cankers came.							
North	N. E.	N. W.	South	S. E.	S. W.	East	West
19	17	18 + (1)*	15	18 + (1)	19 + (1)	33 + (21)	24 + (15)

these wounds it will be seen that there are more cankers on the east and west sides. We are unable to advance a plausible explanation for this.

TABLE 4—*Part of tree infected.*

Year	1911	1912	1913	1914	1915	Total
<i>Location</i>						
Base	1	4	7	15	45	72
Body		2	4	28	42	76
Stub			6	19	20	45
Number			17	22	3	42
Limb	1		3	7	9	20
Branch	1		1	12	27	41
Twig				1	10	11
Top	1	1	2	6	9	19
Root				2	1	3

Base = Infection near ground level;

Body = Below first main divisions of trunk;

Stub = Limb stub on any part of tree;

Number = Point where numbers are nailed on;

Limb = Divisions three inches or more in diameter;

Branch = One-half inch or more in diameter;

Twig = Less than one-half inch in diameter;

The segregation in table 4 is made on the basis of the first or oldest infection on each tree.

TABLE 5—*Infections among trees within ten feet of a diseased tree.*

Date of Infection of diseased trees	No. of trees within 10 ft.	Number of these trees infected each year					No. apparently infected June 30, 1916.
		1912	1913	1914	1915	1916	
1911	6		3	3			0
1912	13			5	2		6
1913	38			9	5	2	22
1914	79				11	2	66

The last examination of the trees was made on June 30, 1916. Relatively few of the 1916 infections would be found in a hasty search made as early as that date.

TABLE 6—*Growth of large cankers vs. small cankers and growth of cankers on grafted vs. ungrafted trees.*

Sept. 1, 1915–Nov. 1, 1915.			Nov. 1, 1915–June 20, 1916.		
No. of cankers	Increase in diameter vertical	horizontal	No. of cankers	Increase in diameter vertical	horizontal
Cankers over 4 in. in vertical diameter					
52	1 64 in.	1.15 in.	68	2 83 in.	2 57 in.
Cankers 4 in. or less in vertical diameter					
21	1 82 in.	1 06 in.	27	2 8 in.	2 33 in.
Growth on grafted trees					
22	1.4 in.	1 2 in.	28	2 61 in.	2.3 in.
Growth on ungrafted trees					
30	1 59 in.	1.2 in.	49	3. in.	2 83 in.

The growth of large cankers as compared to the growth of small cankers was determined for a small number of trees in the fall of 1915. A similar comparison of growth rates was made for cankers found in 1915 and measured on Nov. 1, 1915 and again on June 20, 1916. These data are tabulated in table 6. The amounts of growth made by the large and by the small cankers as shown in this table do not agree but seem to indicate that rate of growth is not greatly affected by the size of the cankers.

A segregation of the same figures was made to compare the relative growth of cankers on native trees with the growth of similar cankers

on grafted trees. Cankers below the graft line of grafted trees were eliminated. These data, as shown in table 6 seem to indicate that cankers grow slightly faster on native trees than on grafted trees.

TABLE 7—*Chestnut blight in grafted and in ungrafted trees.*

	1911	1912	1913	1914	1915	Total No. inf.	Total No. trees	Per cent of trees infected.
Grafted	1	1	3	32	51	88	821	10 27
Ungrafted	1	2	19	26	26	74	306	24 2

Of the total of 1,559 grafted trees, a total of 149 or less than 10 per cent became infected. Of the 829 ungrafted trees, a total of 175 trees or 21 per cent became infected. Data for part of the orchard was segregated by years, showing the erratic proportions given in table 7. Ten grafted trees died from chestnut blight. These trees averaged $5\frac{1}{2}$ inches D. B. H., and died in an average of 2.2 years after the date of infection.

42 ungrafted trees died from the disease. These trees averaged 4 inches D. B. H., and died on an average of 2 years after the date of infection. A few trees were destroyed before notes were taken as to whether or not they were grafted. No distinction was made between cankers above and below the graft line in making these tabulations.

TABLE 8—*Effect of spraying for chestnut blight*

	1911	1912	1913	1914	1915
Number of healthy trees at beginning of year.	2526	2522	2514	2460	2194
Per cent infected during year.	.16	.28	1.6	4.5	7.6
Number of trees sprayed			1550	1493	1424
Per cent of sprayed trees infected during spray season.			1	2.7	4.3
Number of trees unsprayed			964	967	662
Per cent of unsprayed trees infected.			2.3	6.7	7.8

Some trees were cut down in 1914 and 1915. Of the 2,194 trees unblighted at the beginning of the year 1915, 108 were cut down before the end of the season. A number of these felled trees had blighted and all are included in the grand total. They are not included in the totals of sprayed and unsprayed trees. The benefit of spraying was so slight that any protection afforded would seem to be very transitory. Omitting such infections of sprayed trees as were calculated to have occurred before or after the spraying season for each such tree, the results of

spraying as shown by table 8 are still very poor. Extending the length of the spraying season could not be expected to greatly improve results. Trees became infected even when sprayed every fifteen days or when sprayed after each rain. The foliage of lime-sulphur sprayed trees was injured but this injury was not noticeable a few days after spraying. Bordeaux sprayed trees had a characteristic dark blue-green foliage and lost their foliage a week or ten days earlier than lime-sulphur sprayed trees. There seemed to be little choice between the two sprays from either the standpoint of disease control or of effect on the host. Neither spray produced any benefit commensurate with the cost.

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FIELD CONTROL OF TOMATO MOSAIC

MAX W. GARDNER AND JAMES B. KENDRICK¹

WITH ONE FIGURE IN THE TEXT

The importance of the perennial weed relatives of the tomato as winter carriers of the mosaic disease has been pointed out in our previous publications² and the eradication of the perennial ground cherries (*Physalis subglabrata*, *P. virginiana*, and *P. heterophylla*) and horse nettle (*Solanum carolinense*) in and near tomato plant-beds and fields has been recommended as a mosaic control measure in Indiana.

During the summer of 1922 a cooperative trial of these recommendations on a field scale was carried out in cooperation with Mr. Ralph Kemp, a tomato grower and canner. The work was located near one of Mr. Kemp's factories at Kempton, Indiana, in a section of the corn belt very favorable for the production of canning tomatoes and likewise a section where the mosaic disease is annually prevalent to a serious extent, presumably because of the abundance of the weed carrier, smooth ground cherry (*P. subglabrata*).

The tomato plants were grown in a series of 24 plant-beds and two field beds which were located in what was previously a sod pasture in order to obtain as great freedom as possible from the disease-carrying weeds, since these are abundant mainly in cultivated soil. The plants were transplanted to three fairly well separated fields, not far from the plant-beds. Field 2 consisting of five acres and field 3 consisting of 30 acres were planted about the third week in May, and field 1 consisting of ten acres was planted about two weeks later.

A careful search for the mosaic-carrying weeds was made in and around the plant-beds on May 11 and none were discovered. On May 22, however, a number of young *Physalis subglabrata* plants were found in three areas near the plant-beds but none showed mosaic.

Upon this first appearance of the weeds, the area comprising the plant-beds and fields 1 and 2 was surveyed once a week and a vigorous campaign to keep down the weed carriers in and around fields 1 and 2 was begun. No effort was made to keep the weeds out of field 3, which was to serve as a check.

¹ Contribution from the Botanical Department, Purdue University Agricultural Experiment Station, LaFayette, Ind.

² Gardner, Max W. and Kendrick, James B. Overwintering of tomato mosaic. Bot. Gaz. 73: 469-485. 1922.

—————Tomato mosaic. Purdue Agr. Exp. Sta. Bull. 261: 1-24. 1922.

On May 29, *P. subglabrata* was appearing in abundance and about a hundred, of which 12 showed mosaic, were cut down about fields 1 and 2. A few appeared in the plant-beds but did not show mosaic. A week later a still larger number of the weeds had appeared and hundreds of the *P. subglabrata*, including 69 cases of mosaic, were cut out in and around fields 1 and 2, particularly in low areas. One colony of *Solanum carolinense* and later a few *P. heterophylla* plants were also destroyed. At this time it was necessary to clean up only the edges of the fields and the fence rows since the fields were being thoroughly cultivated. An attempt was made to keep the weeds out of a zone 100 feet wide, around the fields. It was found useless to pull the weeds by hand and the hoe was used.

The *Physalis* eradication work was repeated on June 14, June 20, June 28, and June 29. An abundance of new shoots was found each time and a continual effort was necessary to keep them cut down. In addition it became evident early in June that horse cultivation could not be depended upon to destroy the *Physalis* between the tomato rows and it was necessary to have men go over the fields with hoes at least once a week. This campaign was continued until July 19 in field 2, and until early August in field 1. Eradication of the *Physalis* proved extremely difficult because of its great abundance and the ease with which younger shoots escaped detection and also because new shoots were sent up promptly from the rootstocks after the older shoots were cut off. There was a very considerable percentage of mosaic among the weeds.

In the meantime, field 3 received ordinary cultivation and no special effort was made to combat the *Physalis* which was equally as abundant as in the other fields and likewise showed a considerable percentage of mosaic.

The first mosaic on tomatoes was found on July 5, five weeks after it was noted on *Physalis*. A careful inspection of field 2 revealed 12 cases of tomato mosaic among the 18,400 plants in the field or 0.06 per cent. These cases were rogued out, but later no attempt was made to rogue the diseased tomatoes. The results of subsequent surveys to ascertain the relative prevalence of mosaic in the two fields (1 and 2) in which an attempt was made to eradicate the weed carriers as compared with the field (3) in which no such effort was made are shown in table 1.

These results show that eradication of the weed carriers exerted a distinctly retarding effect upon the inception and development of the tomato mosaic epiphytotic, since the infection in field 2 was less than

TABLE 1—*Effect of weed eradication on tomato mosaic*

Date Examined	Field No 1 Weed carriers eradicated		Field No 2 Weed carriers eradicated		Field No 3 Weed carriers not eradicated	
	No of plants examined	Per cent mosaic	No of plants examined	Per cent mosaic	No of plants examined	Per cent mosaic
July 5			18 400	0 06		
July 19	22 105	0 12	18 400	0 4		
Aug 8	10 800	0 33			5 424	7 0
Aug 16			6 800	1 0		
Aug 29	6,400	0 43	3 600	1 8	1 720	14 2

two per cent by the last of August while in field 3 it was 7 per cent on August 8 and 14 per cent the last of the month. An even more striking control was obtained in field 1, which however was planted about two weeks later than the other two fields. In this field there was only one-half of one per cent of mosaic the last of August. These results are summarized graphically in figure 1.

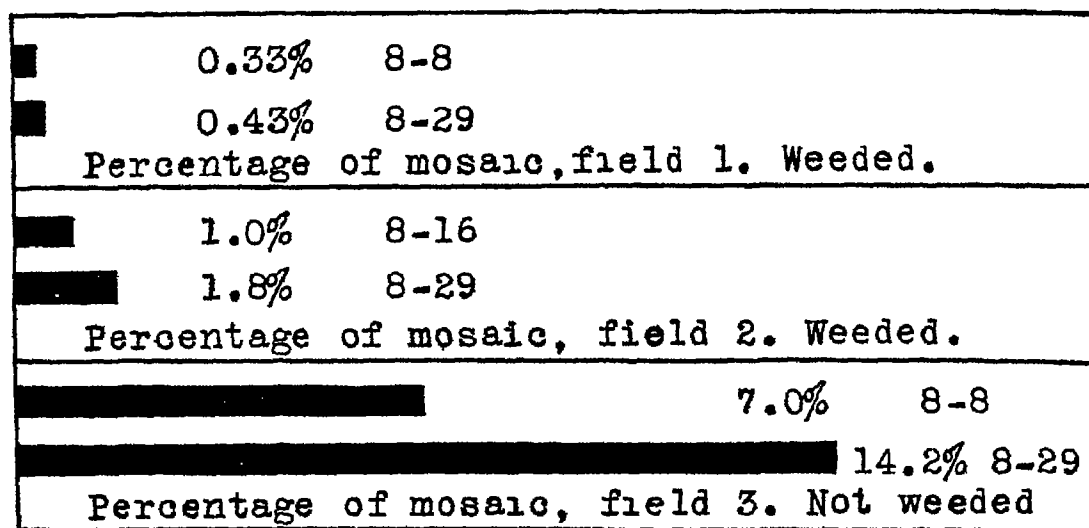


FIG 1 Tomato mosaic control by suppressing the weed carriers

Since these fields produced throughout September there was no doubt a general spread of mosaic after this data was obtained but it is to be remembered that it is the mosaic of early inception which causes the greatest loss.

To summarize this attempt to control mosaic in the field, it may be said that eradication of the mosaic-carrying perennial weeds in and near the plant-beds early in the season and in and around the fields during June and July apparently held tomato mosaic in check. Many difficulties are met with in a weed eradication campaign of this sort, chief of which is the necessity of repeating the process every few days because of the promptness with which new shoots appear. Where the weeds are as abundant as in the tomato regions of central Indiana, many shoots may be overlooked and thus remain a source of infection. It seems entirely feasible for plant growers to destroy these weeds in and near the plant-beds and it is indeed highly desirable to wage an active campaign against them in and around the fields during the early part of the season. In the canning crop it hardly seems advisable to recommend roguing out the early cases of mosaic tomatoes, and it appears desirable to focus attention for the present upon the eradication of the weed carriers.

A means of total eradication of such weeds is much to be desired. In this connection it is of interest to note that in a field near Frankfort in which *Physalis subglabrata* had occurred plentifully in 1919 when it was cropped to tomatoes, in 1920 when it was cropped to corn, and in 1921 when it was cropped to oats and seeded to clover, very few of the weeds could be found in 1922 when the field produced clover hay. No doubt the rootstocks were still alive, however, but the weeds appeared only in spots devoid of clover such as those marking the location of grain shocks.

As to the persistence of mosaic in the weed carriers, rootstocks of mosaic *P. subglabrata* which were planted in a sunken frame in a garden in 1920 and which sent up mosaic shoots in 1921 as previously reported (l. c.), again sent up mosaic shoots in May, 1922. Furthermore, a rootstock of mosaic *P. heterophylla* planted in 1921 sent up a mosaic shoot in May, 1922. In the field near Frankfort, mentioned above, which had not been in tomatoes since 1919, a few cases of mosaic *P. subglabrata* were found in 1922.

Observations made in 1922 further corroborate our previous observations in regard to the relation of the weed carriers to tomato mosaic (l. c.). The weeds occur abundantly in the fields and are very commonly affected with mosaic. They have also been found commonly in and about plant-beds. *Physalis subglabrata* and *Solanum carolinense* seem to be the principal offenders, the former predominating in the richer soils of central Indiana and the latter in the poorer soils of southern Indiana.

CAUSE OF SUNSCALD OF BEANS

H. G. MACMILLAN

A scalding of the pods and other parts of the bean plant (*Phaesolus vulgaris*) as a result of exposure to the sun was noted by the author (5) in 1918. No constituent of the sun's light was at that time designated as responsible for the scalding. The yearly recurrence of the scald has stimulated further study of this problem. Experiments were made during the winter of 1922 to determine, if possible, what factor or factors caused the scald. Bean plants were grown in the greenhouse and subjected to artificial heat and light.

HEAT

Heat is a property of the sun which might cause the scald. It injures beans. MacMillan and Byars (6) observed injury to beans caused by heat estimated to have reached a temperature of at least 54.4° C. No scalding was observed at the time.

In order to determine the effect of mild heat, the ripening pods of beans were exposed to heat radiated from an electric flatiron. Thermometers were placed beside the pods in such positions as to receive an equal amount of heat. No effect of the heat was observed on the pods until the temperature of the thermometer bulb had reached 55.0° C., and had been maintained for 0.5 hour. The effect was not immediately observed but appeared after 24 hours. The surface was dried, white to creamy white in color, and eventually much shrunken. No brown or discolored areas appeared at any time. The unaffected portion of the pod remained as before and reached maturity in due time. No symptom, however, slight, appeared on the heated pods which at any time resembled the true sunscald.

LIGHT

The harmful effect of light on plants has been noted frequently. Maquenne and Demoussy (7) exposed leaves of ivy and fig to the unprotected light of a mercury vapor lamp for two hours and obtained a browning and finally a blackening of the epidermis of the exposed tissue. They estimated that death of the epidermal cells occurred after 15 minutes' exposure to the lamp because of the failure of these cells to react to plasmolysing solutions. Stoklasa (10) repeated these experi-

ments and obtained a similar result. Other investigators have found plant tissues injured by intense light. A great many writers have discussed the effect of ultra-violet light on cellular life in the sterilization of water.

The mercury vapor lamp is presumably the most successful lamp for the ordinary generation of light nearest approaching solar radiation in quality. As no equipment of this kind was available, an arc light of simple construction was employed in the following experiments. A wooden frame was made to hold two $\frac{5}{8}$ inch cored carbons and permit their temporary adjustment in any fixed position. The ordinary 110 volt alternating electric current was used as a source of energy. The strength of the current was lessened by a rheostat composed only of two pieces of common iron baling wire, each 50 feet long, wound into spiral form. The carbons were boiled for 1.5 hours in a concentrated solution of ammonium-molybdate to increase the quantity of ultra-violet light omitted. With fairly constant attention this apparatus gave excellent service, producing a steady light of considerable intensity. The quality of the light is not known, though Schunck (9) says carbons treated as these were emit an abundance of light as short as λ 2,300. In using the light the eyes were always protected by dark glasses. Positive sunburn to the face and arms resulted from an exposure of 0.5 hour at the ordinary working distance.

The bean pods were exposed to the arc light when they had reached a point in their development fairly free from chlorophyll. On a few the peduncle end of the pod showed a green color. This is the stage of development at which the pods show scalding in the field. The pods were exposed to the light at a distance of 25 cm. for periods of 0.5 and 1.0 hours. It was necessary to choose a time when the greenhouse was cool and the ventilation good, in order to maintain a suitable low temperature in the immediate neighborhood of the pods. The arc generates a great amount of heat. On such occasions the temperature rose to only 25° C. at the surface of the pods, as indicated by thermometers exposed near to the pods.

A change in the appearance of the pods was observed 24 hours after exposure, being fainter at first on the pods exposed for only 0.5 hour. A faint browning and streaking appeared, which gradually increased in intensity during the following 72 hours. It was identical in every respect with the normal sunscald, as previously described (5). Bean plants with ripening pods exposed to the full sun for 5 hours each on March 30 and 31 developed mild sunscald. The lesions on the artificially and naturally scalded plants were identical in every respect.

Microscopic examination showed the epidermal cells to be affected. They had become filled with a brown pigmented substance but appeared unharmed in other respects. On the thoroughly whitened pods the stomatal guard cells, which now contained no green matter, were also affected. The cells did not plasmolyse when treated with strong or sugar solutions.

At the same time pods were exposed to the arc light similar pods were likewise exposed, but under the protection of a pane of ordinary window glass. No scald appeared in these pods at any time. At the time the pods were exposed to the sun on March 30 and 31, other pods were similarly exposed, protected by glass, and on them no scald appeared. Ordinary glass is known to be absorptive of light of very short wave length.

The leaves of the plants exposed to the arc light received a similar injury to that of the pods. The epidermal cells of the lower surfaces were killed and colored a deep brown. The cells of the upper epidermis were not so strongly affected. The cells containing chlorophyll were stimulated to increased chlorophyll production and became a deep green. Gradually, however, the leaves began to change color. As the epidermis was killed the leaf slowly lost water. It became unevenly desiccated. The appearance was peculiar in that there were many shades of green in no definite relation with regard to position on the leaf or intensity in color. The leaf was mottled, but not with the definiteness of the mottling of bean mosaic. In three weeks' time the leaf had become well dried and assumed a more general uniform color, though it had not fallen from the stem.

The appearance of the artificially scalded leaves was similar to one always observed on a small percentage of bean leaves in the field at about the time when sunscald of the pods appeared. Heretofore there has been no suitable description for it, and no cause could be assigned. It had mostly the appearance of some deviation from mosaic. It is now apparent that the leaves were suffering from a natural scalding which acted very mildly and over a comparatively long period of time.

The stems and petioles were likewise browned, having the exact appearance of scalded stems as found in the field under natural conditions.

DISCUSSION

The burning of plant cells by chemically active light rays, or light of short wave length, has not been much observed in nature. A thick atmosphere, especially when holding a large amount of suspended

water vapor, is so absorptive of light of very short wave length as to practically prevent its reaching the earth. That some light of short wave length does penetrate to the lowest levels is attested to by the fact that in the tropics cataract of the eye due to coagulation of the epidermal layers of eye tissue is frequently encountered among elderly people (4), although certain conditions of health may predispose the eyes to cataract. In this case, however, the effective wave length is apparently above λ 2,650.

The Colorado bean section, at an altitude of 4,700 feet (1,430 meters) and with a relative humidity of less than 20 per cent for a few hours during the heat of the day, would receive a much larger portion of ultra-violet light than those sections at an altitude varying between sea level and 800 feet (244 meters) and with a relative humidity of from 60 to 80 per cent. Brandenburg (3, p. 862) says that the prevailing lack of moisture in Colorado air is favorable to increased intensity of the direct rays of the sun. Solar temperatures obtained from a black-bulb thermometer in vacuo, exposed to the direct rays of the sun at midday, average during the different seasons about 60° F. higher than the air temperature.

The difference in altitude between western and eastern bean-growing districts may make the sunscald definitely apparent only in the West.

The physiology of the action of ultra-violet light on individual plant cells seems not to be thoroughly understood. Maquenne and Demoussy (8) endeavored to determine this point by comparing the phenomenon shown in leaves dipped in water at different high temperatures, in alcohol, ether, and chloroform vapors, and injured mechanically, with that in leaves sprayed with ultra-violet light. They concluded that the brown color is caused by the action of diastase within the cell acting on the starches which have been released upon the death of the cell. Agulhon (1) finds that ultra-violet light destroys not only the cells but also the active products of the cell. The visible light is active on diastase in the presence of oxygen; the ultra-violet light is able to decompose water, form hydrogen peroxid, and then, in the presence of either free oxygen or only hydrogen peroxid, destroy the diastases by the phenomena of oxidization. Stoklasa (10) says that the browning of leaves exposed to ultra-violet light appears after the protoplasm of the epidermal cells is killed by the chemical action of light. Bovie (2) subjected egg white and ox serum to ultra-violet light in a quartz tube. A coagulum was formed which was similar in all respects to a coagulum formed by heat in these same proteins.

CONCLUSIONS

The sunscald of bean pods is caused by light of short wave length. It kills the epidermal cells of the pods.

The leaves of bean plants may be scalded, resulting in indeterminate symptoms similar in some respects to mild mosaic.

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STUDIES ON THE PHYSIOLOGY OF SOME PLANT PATHOGENIC BACTERIA; VII. PECTIC FERMENTATION IN CULTURE MEDIA CONTAINING PECTIN

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The thin central membrane or middle lamella of cell walls is constituted of a group of substances known as pectic substances. These pectic substances are found in practically all parts of green plants but are relatively most abundant in young rapidly growing parts, in fleshy roots, as beets, turnips, and artichokes, and in succulent fruits as apples, oranges, pears and currants. The common property of causing the gelatinization or jellying of fruit juices is in the popular mind, associated with pectic compounds, but they are of special interest to the plant pathologist by virtue of the fact that invading organisms can pass between host cells only after having attacked these substances, presumably by means of pectic enzymes.

It is to various biochemists that credit is due for a knowledge of the constitution of pectic substances and of their cleavage products. This phase of the problem together with an adequate historical treatment of the subject are comprehensively dealt with in a recent investigation by von Fellenberg (3). It should appear from the terminology of these biochemists that the name protopectin (pectose of older writers) is applied to the complex mother substance from which the simpler pectic compounds are derived. The protopectin of unripe fruits is insoluble but through the action of the enzyme pectosinase, during ripening, it is transformed into soluble pectin. Soluble pectin may then be coagulated to a gel by pectase, provided calcium or some other suitable base is present, and this gel then be hydrolyzed by pectinase, to reducing sugars or pectinase may hydrolyze pectin directly. According to von Fellenberg (3), the pectin complex is composed of mucic acid forming groups, methyl pentose, arabinose, carboxyl groups and methyl alcohol. The cleavage products could, therefore, be expected to serve well as a source of supply of carbon compounds for certain organisms.

¹ Previous publications in this series have been compiled in Technical Bulletin 20 of the North Carolina Agricultural Experiment Station.

Phytopathological research may be credited with having clearly shown, or at least strongly postulated, the possession by a number of fungi and bacteria, of the property of secreting pectic enzymes. The organism causing brown rot of stone fruits, *Sclerotinia cinerea*, may be cited as an example of the fungus which effects the decomposition of tissues by dissolution of the middle lamella and then assimilates the pectin in its own metabolism (4). Among the plant pathogenic bacteria, the soft rot of certain vegetables, *Bacillus carotovorus* (2) and the black rot of crucifers, *Bacterium campestre* (5) have no doubt been most thoroughly investigated as to their pectin relations.

It may be stated, without including an extensive review of literature on technique, which is not pertinent in this paper, that several methods have been used to demonstrate pectic enzyme activity by fungi and bacteria. In view of our limited knowledge of the quantitative activity of enzymes, all methods perforce are qualitative, some rather laborious, and none well adapted to the detection of weakly acting pectin ferments. Further, it does not appear that use has been made in any foregoing investigation of culture media containing pectin to determine this physiological property. A method for its preparation will, therefore, be described, data on its use recorded and a brief discussion accorded it.

Methods. A commercial product known as "Certo" prepared by the Pectin Sales Co., Rochester, N. Y., was used in the preparation of relatively pure pectin. The method employed was essentially like that used by Caldwell (1). To this Certo, an equal volume of 95 per cent alcohol was added which caused the precipitation of the pectin as a gelatinous fibrous rubber-like mass. The brown coloring matter of the Certo for the most part remains in the alcohol. This mass of pectin was collected on a cheesecloth in a porcelain filter, the alcohol worked out of it with a spatula and it was then washed with small quantities of fresh alcohol. To further purify the pectin, it was dissolved in as small a quantity of warm water as would secure complete solution and reprecipitated with an equal volume of 95 per cent alcohol. The mass of pectin was again washed, pressed out and washed after which it was permitted to dry slowly at a temperature of 70° C. No coloring matter remains in the alcohol of the third precipitation. The dry pectin thus prepared is a whitish gray mass acidic in character which may be easily pulverized and dissolves readily in warm water.

Bacto-agar containing 18 grams to 100 cc of water or plain bouillon containing 1 per cent of Difco peptone, 0.3 per cent Liebig's beef extract and 0.5 per cent NaCl were then prepared as stock culture media. To these media, after they had been flaked, sterilized and cooled to

about 60° C., about 1 per cent by weight of powdered pectin was added which with agitation dissolved. The reaction was then adjusted to pH 7.4 by the addition of strong NaOH solution, and phenol red was introduced into the agar media as an indicator. Aseptic precautions were observed throughout, making unnecessary a final sterilization, which would have changed both the hydrion concentration (6) and the pectin content. The media were then poured into previously sterilized test tubes. After 48 hours' incubation, in order to insure their freedom from contamination, they were inoculated. The following organisms were employed: *Bacillus carotovorus*, *Bacterium tabacum*, *Bact. angulatum*, *Bact. sojae*, and *Bact. campestre*. In the liquid media, changes in reaction were followed by colorimetric readings at definite consecutive intervals.

Results. Cultures of these several organisms in pectin bouillon were first prepared, incubated at 25° C. and the changes induced by each recorded in the following table:

TABLE 1—Changes in reaction in pectin bouillon, initial pH 7.4

Organism	Age of cultures in days and pH concentration Days		
	3	5	7
	pH	pH	pH
<i>Bacillus carotovorus</i>	5.4	5.2	5.2
<i>Bact. tabacum</i>	7.0	6.8	6.4
<i>Bact. angulatum</i>	7.0	6.8	6.4
<i>Bact. sojae</i>	7.0	6.8	6.8
<i>Bact. campestre</i>	6.8	6.4	6.0

Manifestly all of these organisms are able to attack pectin with the formation of acid but of them the soft rot organism exhibits the most vigorous fermentative action. Since it has been shown in another study (6) that when cultured in plain bouillon all cause this medium to become progressively more alkaline except in the case of *Bacillus carotovorus*, in which an increase in hydrion concentration is followed by a reversal of reaction, it is apparent that this increase in acidity comes from the fermentation of pectin. In the light of the findings as to the constitution of the pectin complex, acid production can reasonably be regarded as postulating enzyme activity.

Pectin agar containing phenol red as an indicator was employed in order to check these changes in reaction and provide an ocular demonstration of this progressive change. In this medium, the color had all disappeared, in stab cultures of *Bacillus carotovorus*, within 3 days whereas there was only a slight change beneath the surface of the agar

in the case of the other organisms. By the seventh day, the color had disappeared in stab cultures of cabbage black rot, very pronounced change had taken place in the cultures of the tobacco leaf disease organisms, but only slight change in the case of the soybean blight. The rate of the disappearance of color is thus in accord with the findings in liquid cultures.

Discussion. In the case of organisms which vigorously attack the middle lamella of succulent tissues, as is the case with *Bacillus carotovorus* as demonstrated by Jones (2) and *Bact. campestre* as demonstrated by Smith (5), the solvent action of pectic enzymes is easily demonstrated by any one of several methods. For such, the development of additional methods for the qualitative determination of pectic enzymes may have no special value. For organisms of the type of the others, whose pectin relations are probably like those of the great majority of leafspot disease producers, it is either impossible or difficultly possible, because of their relatively weak pectin decomposition, to demonstrate pectic enzymes. For such, this method is believed to be adapted because of its delicacy since a slight increase in acidity can be both easily and readily measured. It should also facilitate a study of the invasion of host tissues by this type of organism and of the pathological anatomy.

Summary. Pectic fermentation may be postulated in culture on media containing pectin. Increase in acidity is made the criterion on which the presence of pectic enzymes is shown.

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DAMPING-OFF OF TAPROOTS OF CONIFERS

ANNIE E. RATHBUN

Formerly the term damping-off was used to designate primarily the death of a seedling which resulted from the attack of a parasitic fungus at the soil surface; this caused there a local constriction of the stem and later the fall and wilting of the seedling. However, several years ago Büttner (1) recognized that root infections were frequent, while Hartley (2, 3) in his description of normal damping-off stated that in sandy soils root infections are more common than hypocotyl infection and that damping-off in nursery beds is in most cases, primarily a root rot of some type. These conclusions have been based for the most part upon either field observations or artificial infections of coniferous roots produced indirectly by the same methods of soil inoculation employed in most other damping-off and root rot investigations (2, 3). This indirect method does not show for normal damping-off the amount of injury which results respectively from root and from hypocotyl infections; and it does not indicate the relative degree of parasitism of the different fungus species when free from fungous inhibitors and soil. In order to secure some information on these points several small direct inoculation experiments with the taproots of seedlings of *Pinus banksiana* Lamb., *Pinus resinosa* Ait. and *Picea engelmanni* Engelm., have been conducted by the writer. Inoculation was made while the seed coats were still clinging to the cotyledons of the seedlings. The seedlings were grown in sand which had been autoclaved and watered with boiled water. Three very similar methods of inoculation were used (4).

The first method was to cut away one-half to two-thirds of a Petri dish culture; then to place the seedlings in the Petri dish in such a way that the roots were in contact with the inoculum while the stems touched only the glass. Both corn meal and prune agars were used.

A second method was to grow the fungi on rice mush in Erlenmeyer flasks. Several grains of the rice plus the spores and mycelium growing upon them were transferred to the bottom of a sterile Petri dish. Then the seedlings were laid in the Petri dish with the root of each seedling in contact with the inoculum at one point. With this method the seedlings showed a slight tendency to wilt.

In the third method in order to overcome this wilting tendency the bottom of each Petri dish was lined with filter paper moistened in boiled water before the addition of the inoculum. Otherwise this method did not differ from the second.

Control seedlings were treated similarly to the inoculated ones except that sterile rice or agar from the same batch as the inoculated medium, was used. Each control and each inoculated unit consisted of ten seedlings. The duration of each experiment was four days.

Before removing the seedlings from the sand each pot was thoroughly soaked in boiled water. This allowed the seedlings to be removed from the sand more easily and consequently reduced the possibility of injury to the root hairs or small rootlets thus checking much of the physiological disturbance which otherwise might have resulted.

The inoculating fungi in these experiments were *Pythium debaryanum* Hesse, *Corticium vagum* B. and C., *Fusarium* spp., *Botrytis* spp. and certain miscellaneous fungi.¹ Many of them have been tested by Hartley (2, 3) in various indirect inoculation experiments and all of them have been used by the writer in the direct inoculation of coniferous seeds, young radicles, and stems. The results secured from inoculating these last three will be published elsewhere.

DISCUSSION OF RESULTS

The results of the present investigation are given in table 1. On account of the small number of experiments and the failure to secure distinctly different results from the different methods, it was thought best to present merely a summary of the results instead of comparing the relative virulence of the various strains on the different media. The fact that the controls of *Pinus banksiana* and *P. resinosa* and in all but one experiment, those of *Picea engelmanni* always remained free from rot indicates that the rots observed were really parasitic effects and not consequent upon the removal of the seedlings from the soil.

Pythium Debaryanum

Pythium debaryanum is very parasitic to the taproots of *Pinus banksiana*, *P. resinosa* and *Picea engelmanni*. There is apparently, so far as these experiments show, no significant difference in the susceptibility of the three species. The tested strains seem to exhibit approximately equal virulence whether they were isolated from coniferous or other hosts. Moreover, except for one case with each of two strains they were always of approximately equal high virulence. This seems to indicate that some other factor, such as inability to maintain themselves in the soil until the seeds had germinated, contributed to the difference in the virulence of these same strains reported by Hartley (2, 3). It is very possible if a larger number of strains had been tested in these

experiments, a greater range in virulence variability would have been secured.

Corticium Vagum (Rhizoctonia solani Kuhn).

Many of the *Corticium vagum* strains were used in only one experiment while others showed great variability in their ability to attack coniferous taproots. Two strains were consistently highly virulent and two others were consistently non-parasitic to taproots. On the whole, *Pinus resinosa* seemed more resistant than the other two hosts to this fungus. However, further experimentation is necessary to establish this point. The reason for the apparently greater resistance of *Pinus resinosa* may be the fact that fewer *Corticium* strains were used on it than on either of the other hosts. As far as these experiments show the strains isolated from coniferous hosts were not significantly more parasitic to taproots of conifers than the strains isolated from other hosts.

Fusarium spp.

The single tested strains of *Fusarium sporotrichioides* Sherb., and *F. arthrosporioides* Sherb., apparently equalled the most virulent strains of *Pythium debaryanum* in parasitism to taproots. *Gibberella saubinetii*? (Mont.) Sacc. and species of the moniliform section of *Fusarium* were moderately parasitic. There is no evidence to prove that the strains of the latter isolated from coniferous hosts were more parasitic than those isolated from other hosts. The other species of *Fusarium* were either doubtfully parasitic or non-parasitic. There is some indication, which needs confirmation, that *Picea engelmanni* is less resistant than the pines to some of the *Fusarium* species.

Miscellaneous Fungi

Rheosporangium aphanidermatus Edson equalled *Pythium debaryanum* in ability to attack taproots. The large sclerotial Botrytis, commonly called *B. cinerea* Pers., was decidedly parasitic to the taproots of *Pinus banksiana*, but gave less evidence of virulence when used on *Pinus resinosa*. *Phomopsis juniperovora* Hahn was able to attack the taproots of *Picea engelmanni* and *Pinus resinosa* while *Phytophthora* sp., was able to attack the latter. *Rhizoctonia potomacensis* Wr., which may be only a strain of *Corticium vagum*, gave some slight evidence of parasitic ability while *Pythium artotrogus* (Mont.) de Bary and *Mucor racemosus* Fres. showed no evidence of parasitism.

	Pinus banksiana				Pinus resinosa				Picea engelmanni			
	Number of strains	Number of seedlings inoculated	Number of seedlings decayed	Percent of seedlings decayed	Number of strains	Number of seedlings inoculated	Number of seedlings decayed	Percent of seedlings decayed	Number of strains	Number of seedlings inoculated	Number of seedlings decayed	Percent of seedlings decayed
Inoculating fungus**												
Phythium artotrogus	1	10	1	10	1	10	1	10	—	—	—	—
Fusarium culmorum group (a, b)	3	30	0	0	—	—	—	—	3	30	0	0
Fusarium elegans group (a, b)	1	10	0	0	5	50	5	10	1	10	10	100
Fusarium solani (Mart) Ap. and Wr.	1	10	0	0	—	—	—	—	1	10	9	90
Fusarium between discolor and roseum groups (b)	1	10	0	0	1	10	2	20	—	—	—	—
Fusarium sporotrichioides (a)	—	—	—	—	—	—	—	—	1	20	20	100
Phomopsis juniperovora	—	—	—	—	2	20	15	75	1	20	14	70
Phytophthora sp.	—	—	—	—	3	30	14	47	—	—	—	—
Fusarium hyperoxysporum Wr.	—	—	—	—	1	10	3	30	—	—	—	—
Fusarium vasinfectum Atk.	—	—	—	—	1	10	0	0	—	—	—	—
Fusarium ventricosum Ap. and Wr.	—	—	—	—	1	10	0	0	—	—	—	—
Mucor racemosus	—	—	—	—	1	10	0	0	—	—	—	—
Controls	—	400	0	0	—	175	0	0	—	350	30	9*

* Three control plates infected in one experiment which was badly overgrown with *Mucor* sp.

** Fungi arranged in order of apparently decreasing virulence to *Pinus banksiana*.

(a) Identified by Miss Helen Johann.

(b) Identified by Dr. C. B. Sherbakoff.

(c) May be a strain of *Coritium vagum* according to H. A. Edson who furnished the culture.

The fact that with the exception of *Fusarium arthrosporioides*, no fungus which has not previously been reported (2, 3, 5) as a potential damping-off parasite caused in these investigations serious decay of taproots, seems to indicate that these methods of inoculation really do show which species are likely to be most virulent in the field. However, it apparently shows that some strains especially of *Pythium debaryanum* were formerly considered slightly or non-parasitic because of their inability to maintain themselves in the soil or to combat fungus inhibitors until the seeds had germinated. Even these less virulent strains would possibly do considerable damage in nursery beds if they happened to infect the soil at a time when the taproots were young. *Pythium debaryanum*, *Corticium vagum*, *Fusarium sporotrichioides*, *Botrytis cinera* and *Phomopsis juniperovora* have previously (5) been reported as causing, under artificial conditions, more or less serious root rot of pine seedlings too old to succumb to the more ordinary types of damping-off. The same species have been found virulent on young stems and radicles, while both *Mucor racemosus* and *Pythium artotrogus* have been found to be non-parasitic. Further experimentation is necessary to definitely establish the relative susceptibility of the taproots of the above hosts as well as the relative virulence of the tested fungi.

SUMMARY

1. These direct inoculations of taproots of very young coniferous seedlings in Petri dishes seem to indicate that differential results can be secured by the use of these methods.

2. *Pythium debaryanum*, *Rheosporangium aphanidermatus*, *Fusarium arthrosporioides* and *F. sporotrichioides* are very virulent, under the artificial conditions of these experiments, to the taproots of young conifers. Since the last three, despite the large amount of diagnostic work recently reported (3), have never been found naturally infecting coniferous seedlings, *P. debaryanum* is considered the most dangerous parasite of coniferous taproots.

3. *Corticium vagum*, *Botrytis cinera*, *Phomopsis juniperovora*, *Phytophthora* sp., and strains of the moniliform section of *Fusarium* are apparently able to cause considerably decay of coniferous taproots.

4. *Mucor racemosus*, *Pythium artotrogus* and certain *Fusarium* species are apparently non-parasitic to coniferous taproots.

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The cultures used in these experiments belonged to Dr. Carl Hartley. He obtained them from D. Atanasoff, M. F. Barrus, H. A. Edson, L. L. Harter, L. A. Hawkins, G. N. Hoffer, Mrs. C. R. Tillotson, H. H. Whetzel and various members of the Office of Forest Pathology. Miss Helen Johann identified Mr. Atanasoff's culture and Dr. C. B. Sherbakoff named several of the other *Fusarium* cultures. The writer wishes to express her thanks to the above people for their kind assistance and especially to Dr. Hartley for valuable suggestions.

THE RELATION OF SOIL MOISTURE TO FORMALDEHYDE INJURY OF ONION SEEDLINGS

P. J. ANDERSON

WITH TWO FIGURES IN THE TEXT

In controlling onion smut by the application of formaldehyde in the furrow, only fairly dilute solutions may be used. If concentrated solutions are applied, even though the actual quantity of formaldehyde per unit of row is no greater, the seeds fail to germinate. Almost from the beginning of the practice of controlling smut in this way, it has been customary to dilute the solution by adding 1 pint of the commercial formaldehyde (said to be a 40 per cent solution of the gas in water, but usually analyzing less) to 16 gallons of water, thus making a solution containing 1 part of formaldehyde to 128 parts of water. The standard recommendation now is to apply this dilute solution at the rate of 16 gallons to about 3000 feet of drill. For convenience in discussion and for ready comparison with other rates of dilution and application, this standard may be called "the 1-128-3000 formula." Although smut can be controlled by the use of this formula, the practical onion grower finds certain serious objections to the use of so much water. It makes the soil so wet that the seed sprout, packing wheel or coverers frequently become clogged with mud. A more serious objection is the extra labor involved in drawing so much water, in more frequent refilling of the tank and particularly the increased weight of the drill when so much water must be pushed across the field. Inquiry among the growers of the Connecticut Valley onion region brought to light the fact that, although the dilution of 1 part to 128 had been recommended by the experiment stations for many years, none of them were using it. They preferred to use a smaller quantity of a more concentrated solution even at the risk of killing the seed. Recognizing the futility of recommending any longer a formula which the practical grower would not use, the Department of Botany of the Massachusetts Agricultural Experiment Station started investigations in 1918 with the object of determining whether the extreme dilution of the 1-128-3000 formula was necessary, and if not, to find what formulas involving less dilution could be safely used. The results of the investigation as regards the control secured by use of formulas involving more concentrated solutions will be presented in a future publication: the present paper will be devoted to a discussion of some phases of formaldehyde injury.

During the first years of the field investigation it was found that concentrated formalus such as 1-64-3200 and 1-50-3000 could be safely used with but slight apparent reduction in the percentage of onions which came up. When, however, in 1921, the same formulas were used on the experimental fields, they caused very decided loss in the number of seed which sprouted as compared with the adjacent untreated rows. Practically all of the formulas tried caused more or less injury. Many growers sustained so much loss from the use of formaldehyde that they plowed up the fields and planted other crops. We were at first inclined to believe the trouble was due to a difference in the character of the formaldehyde which was used. Samples of formaldehyde were therefore collected by A. V. Osmun and the writer from sixteen different sources, including among them samples of the same material which we had used during the previous years and which had not caused injury. Chemical analyses of the samples revealed no differences in composition which were of sufficient size or character to warrant a suspicion of their toxic effect. The manufacturers assured us that there had been no change in the method of manufacture of formaldehyde. The most apparent difference was in the percentage of methyl alcohol contained in the various samples. The sixteen samples were next tested simultaneously on onion seeds sown in greenhouse benches, using for each the formulas 1-50-3000, 1-50-5000, 1-100-3000 and leaving untreated rows between the plots. In order to see what effect variation in the percentage of methyl alcohol would have, samples containing known percentages from 4 per cent to 16 per cent alcohol were included in the tests. Without discussing the results in detail, it may be briefly stated that (1) the source of the formaldehyde was without influence on the percentage of plants which came up, (2) variation in the percentage of methyl alcohol made no difference, (3) with all samples and every formula used, formaldehyde *retarded* germination for a day or two and, (4) with all samples and formulas, formaldehyde *prevented* the germination of a certain percentage of the seed, this loss being more apparent when a more concentrated solution was used or where the quantity of the solution was increased. The results of these experiments eliminated the possibility that the losses of 1921 were referable to difference in the character of the formaldehyde.

Since there had been no differences in the formulas or method of application of formaldehyde nor in the treatment of the soil in 1921, there appeared to remain only the character of the season itself as a possible explanation of the trouble. The character of the spring weather could obviously affect the soil—where the injury occurs—in two ways:

(1) in the percentage of moisture and (2) in the temperature. The experiments described below were undertaken with the object of determining to what extent the percentage of injury is influenced by variation in the percentage of moisture in the soil. The effect of the soil temperature on injury has not been determined as yet.

EXPERIMENT WITH SOIL A.

Soil A was a typical sandy onion soil of the Connecticut Valley. The moisture retentive capacity of this soil was found to be 51 per cent of the dry weight of the soil.¹ The experiments with Soil A were conducted during the winter months in the greenhouse in 8-quart glazed pots without drainage openings in the bottom. About an inch of small stones was placed in the bottom of each pot, a weighed amount of sifted soil added and brought up to the desired moisture content by pouring water from above. After settling for a day the soil was worked up thoroughly and 100 seeds planted in a circular row in each pot. The desired quantity of formaldehyde solution was dripped over the seed from a burette and immediately covered. As a check for each treated pot a second pot received the same treatment except that water instead of the formaldehyde solution was dripped over the seed. All pots were brought up to the original weight by addition of water every second day, alternating between surface sprinkling and subwatering through a deep hole in the center of the soil. The percentage of germination was counted at the end of 2 weeks, 3 weeks, and 4 weeks. The results of the two sets of experiments with this soil are tabulated below. (Tables 1 and 2.)

This preliminary experiment indicated that the soil with 11 per cent moisture was too dry for good germination, while that with 40 per cent was too muddy. Leaving these two out of consideration, it was evident from this experiment that the formaldehyde injury was greater in the dry soils and decreased as the percentage of soil moisture increased.

In the next experiment the extremely dry and wet pots were omitted and the intervals in moisture content between pots was diminished. The results of the second experiment are presented in table 2 and represented graphically in figure 1.

¹ The retentive capacity was determined as follows: Two samples of the soil were dried in the electric oven at 105° C. for 24 hours, then sifted through a 1 mm. sieve. One hundred grains of each sample were then placed in celluloid cylinders of 1 inch diameter supported upright on a ring stand and closed at the bottom with filter paper disks held in place by pieces of fine cheesecloth over the bottom. Water was poured into the top of the cylinders until it saturated the soil and dripped from below. After the drip had ceased the soil was found to weigh 151 gms. The same method was used for determining the moisture retentive capacity of all soils used in subsequent experiments.

TABLE 1—*Results of formaldehyde treatment of onion seeds with different percentages of soil moisture*

Percentage of soil moisture	Plants up after 2 weeks			3 weeks			4 weeks		
	Treated	Check	Percent Loss	Treated	Check	Percent Loss	Treated	Check	Percent Loss
11	0	0	0	0	21	100	0	25	100
17	12	84	86	33	84	61	33	86	61
23	10	80	87	35	84	58	40	84	53
28	37	70	47	54	72	25	54	72	25
33	33	64	49	47	68	31	48	68	29
40	19	64	70	37	74	50	38	74	49

This experiment shows more clearly than Experiment 1 that the greatest injury is in the dry soil and that the percentage of injury decreases fairly regularly with the increase in the percentage of soil moisture. Figure 1 also illustrates very clearly the retarding effect of the formaldehyde: the difference between the treated and untreated rows at the end of two weeks (represented by the uppermost broken line) was considerably greater than at the end of three or four weeks. This same retarding effect was noted and measured in all the later experiments, but is not indicated in the tables presented below.

EXPERIMENTS WITH SOIL B.

Soil B had a higher percentage of organic matter and less sand than soil A, therefore a higher moisture retentive capacity. By the method previously described the retentive capacity was found to be 74 per cent. All the succeeding experiments were conducted with soil B. For these experiments, regular galvanized iron soil pots of 30 inch diameter and 14 inch depth were used. Two $\frac{5}{8}$ inch galvanized pipes projected above the soil on opposite sides of pots and were connected below by a perforated half-cylinder arm. Water was added on alternate days by pouring down the side pipes and by sprinkling from above. All pots were kept up to weight throughout these experiments as in the previous ones.

Purposes of the experiments with soil B. By the previous experiments it has been determined that formaldehyde injury varies inversely as

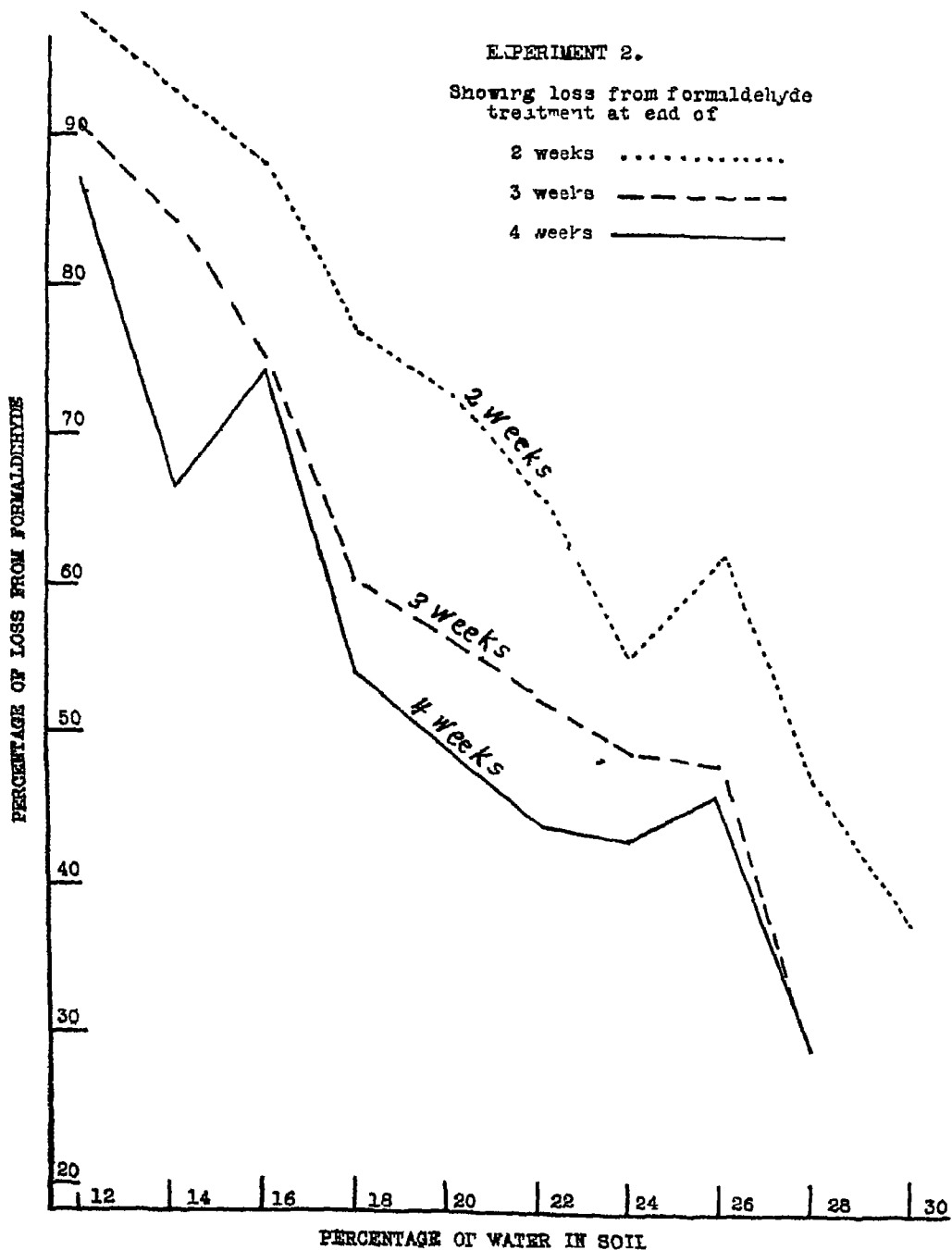


FIG. 1. Graph, showing the decrease in injury with increase in soil moisture.

the percentage of soil moisture when the 1-50-3000 formula is used. It was next desired to find out what the effect would be (1) when the formaldehyde is applied in more dilute solution and (2) when a smaller

TABLE 2—*Results of treatment of onion seed with formaldehyde (Formula 1-50-3000) with different percentages of soil moisture*

Percentage of soil moisture	Plants up after 2 weeks			3 weeks			4 weeks		
	Treated	Check	Percent Loss	Treated	Check	Percent Loss	Treated	Check	Percent Loss
12	1	66	98	6	68	91	10	69	87
14	5	81	93	14	76	84	25	76	67
16	9	79	88	20	80	75	20	80	75
18	19	83	77	34	81	60	37	81	54
20	21	77	73	35	76	54	39	76	49
22	26	76	66	36	77	53	43	77	44
24	34	76	55	40	78	49	44	78	43
26	30	80	62	42	82	48	43	82	47
28	38	72	47	52	71	29	52	71	29
30	49	77	37	55	77	28	55	77	28

quantity of the 1-50 solution per unit of row is applied. To test the first, the standard 1-128-3000 formula was used and for the second, the 1-50-4000 and 1-50-5000¹ formulas were tried. A third object was to see whether a change in the character of the soil would affect the results.

Effect of percentage of soil moisture on germination of the seed. In soil A it was found that the percentage of seed germination fell off rapidly below a moisture content of 12 per cent and above 30 per cent. Germination within the 12-30 limit was fairly constant and was regarded as normal. In soil B, with its higher retentive capacity, the range of normal germination was considerably higher. Considering as normal only those pots in which the seeds germinated to more than 60 per cent

¹ This formula represents approximately the rate of application used by many growers in the Connecticut Valley onion region. They add 1 gallon of formaldehyde to 1 barrel (50 gal.) of water and distribute this amount over 1 acre of onions. This formula is very convenient in practice and probably owes some of its popularity to that fact.

it will be observed from the data in the second column¹ of table 3 that the normal range extended from 24 to 44 per cent. The germination below and above these figures was erratic and uncertain. Their value

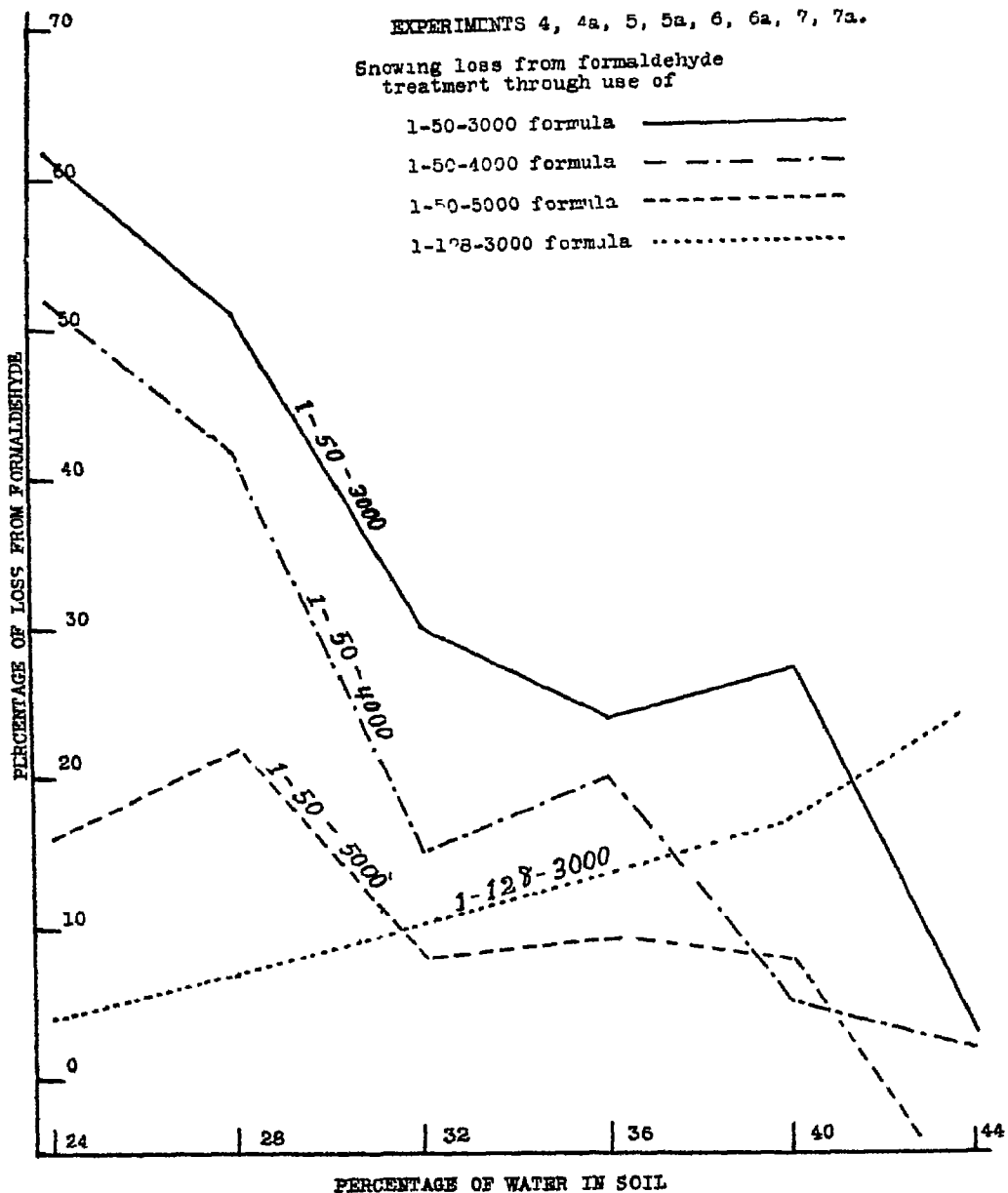


FIG. 2. Graph, showing effect of diluting the formaldehyde solution and of planting when the soil water is low.

for comparison is therefore doubtful. In considering the lack of germination in the treated very dry or very wet pots, there was always

¹ Each figure in this column is the average germination of eight check pots (800 seeds) for this particular soil moisture in the 8 experiments conducted with Soil B.

TABLE 3—Summary of Experiments 4, 4a, 5, 5a, 6, 6a, 7, 7a.

Percent- age water in the Soil	Av. Ger- mination of 8 checks	Exp. 4 + 4a 1-128-3000			Exp. 5 + 5a 1-50-3000			Exp. 6 + 6a 1-50-4000			Exp. 7 + 7a 1-50-5000		
		Av. Germ.	Dif. from av. ck.	Percent loss	Av. Germ.	Dif. from av. ck.	Percent loss	Av. Germ.	Dif. from av. ck.	Percent loss	Av. Germ.	Dif. from av. ck.	Percent loss
16	8	0	8	100	0	8	100	—	—	—	—	—	—
20	28	27	1	4	9	19	61	6	2	25	9	19	67
24	69	66	3	4	26	43	62	33	36	52	58	11	16
28	79	73	6	7	39	40	51	46	33	42	62	17	22
32	71	64	7	10	50	21	30	60	11	15	65	6	8
36	74	64	10	13	52	18	24	59	15	20	67	7	9
40	76	63	13	17	55	21	27	72	4	5	70	6	8
44	68	51	17	25	67	1	2	65	3	3	75	—7	—10
48	51	28	23	45	58	—7	—13	55	—4	—8	56	—5	—9
52	41	8	33	80	50	—9	—22	34	7	17	30	11	27
56	17	5	12	70	42	—25	—147	3	14	83	6	11	65

in the writer's mind a question as to whether the injury was due to the condition of the soil or to the formaldehyde treatment. Although the data for all moisture percentages which were tried is presented in table 3, it is probably safest to use the results of only those between 24 and 44 per cent in drawing conclusions.

Methods for experiments 4, 4a, 5, 5a, 6, 6a, 7, 7a. The four formulas 1-128-3000, 1-50-3000, 1-50-4000 and 1-50-5000, were tested in soil ranging at intervals of 4 per cent from 16 to 56 per cent. For each treated pot there was one check pot of the same moisture content and treated in the same way throughout the experiment except that water instead of formaldehyde was applied to the seed when planted. Each experiment was repeated and each of the figures in table 3 represents the average of duplicate experiments. Percentages of germination were recorded at the end of 2 weeks, 3 weeks and 4 weeks as in the previous experiments, but only the results at the end of 4 weeks are given in table 3. The percentage of loss in each case is computed from the difference between the average germination of the two treated pots and the average of the 8 check pots. It is believed that the average of the 8 gives a more accurate basis of comparison than merely the two which were used for that particular experiment.

Discussion of results. (1) As will be seen by inspection of table 3 and more readily perhaps in the graph (Fig. 2) the results of the experiments with the 1-50-3000 formula confirm our previous conclusion that the amount of injury varies inversely as the moisture content of the soil. The maximum injury was not as high as for the experiments with soil A. This may have been due to a difference in the character of the two soils, but additional experiments are necessary before any certain conclusions on this matter can be drawn. (2) When the amount of the 1-50 solution per unit of row is reduced, as in the 1-50-4000 and the 1-50-5000 formulas, the amount of injury in the dry soils is reduced proportionately. (The effectiveness of the solution for the control of smut is probably reduced in the same ratio.) (3) When the actual amount of formaldehyde per unit of row is kept constant, but the dilution is increased, the injury in dry soils is diminished. Thus with the 1-128-3000 formula the injury in a dry soil is no greater than in more moist soils; in fact the results of experiments 4 and 4a seem to indicate that there is *less* injury in dry soils. This apparent increase in injury with increase in soil moisture may be partly explained in this case by the deleterious effect of the addition of so much water to the more moist soils at the time of planting the seed. This was apparent when these pots were compared with their own checks instead of with

the average of the eight. As far as effective control is concerned these results vindicate the standard 1-128-3000 formula as the safest one to use.

TIME OF THE INJURY

At what time after the seed is planted is the injury produced? This question is of considerable practical importance as will be indicated below. There would seem to be three possible answers: (1) The seed may be injured or killed immediately or within a few hours after planting by coming in contact with a fairly concentrated solution. (2) Injury may result from the repose of the seed for several days in a formaldehyde-soaked soil. (3) The injury may be due to the action of the formaldehyde on the young radicle and cotyledon when they emerge from the seed three or more days after planting. If the first of these three possibilities is the true explanation, then the amount of injury may be predicted from the condition of the soil on the day when the seed is planted, i. e., the weather conditions immediately *preceding* the planting day will determine the amount of injury. If, on the other hand, either of the other two is the true explanation, the percentage of injury may be greatly influenced by weather conditions *subsequent* to the day of planting; e. g., a heavy rain occurring the next day after planting might dilute the formaldehyde to such an extent that injury would be greatly reduced or prevented.

In order to determine this point the following experiment was conducted: Six rows of onions were planted in a greenhouse bench in the usual way. Half of them were treated with formaldehyde according to the 1-50-3000 formula, the other half being left untreated. At the same time on each succeeding day, 100 seeds from the treated rows and 100 seeds from the untreated rows were dug out, washed for an hour in running water to remove formaldehyde and then planted in another part of the bed in new soil. The ground during the experiment was fairly dry, but the percentage of water was not determined. Four weeks after the experiment was started the seedlings which had come up were counted. Table 4 below shows the results:

The average difference in viability between the treated and untreated seeds is 13. The difference was not less when the transplanting was done one day after treatment. There is no evidence here of an increase in injury with the length of time during which the seed remained in the formaldehyde-soaked soil. This experiment indicates then that the injury is caused directly to the seed on the day of treatment.

TABLE 4—*Experiment 3; showing the number of treated and untreated seeds which germinated after washing and transplanting*

Days between planting and digging	Treated	Check	Difference
1	60	76	16
2	49	52	3
3	65	79	14
5	55	73	18
6	58	81	23
7	73	77	4
8	63	75	12

PRACTICAL APPLICATION OF THE RESULTS

When the soil is very dry it is not safe for the grower to use as concentrated a formula as 1-50-3000. If, however, the soil is fairly wet on the planting day, he may save considerable labor and time by using a concentrated formula and at the same time he will sustain no more, or at least little more, loss of seed than if he used a dilute formula like 1-128-3000. With some practice he could probably learn to gauge his formula of application according to the condition of the soil on that day. It is not likely that the weather of succeeding days will influence the results. If the soil is extremely dry and dusty, it is safest to use a dilution approximating the 1-128-3000 formula. Since there is always some loss from the use of formaldehyde, the grower should plan to use more seed per acre than on fields where smut is not present. Some growers have told the writer that they have practised this in past years.

SUMMARY

1. Any application of formaldehyde which is strong enough to control smut also causes a certain percentage of the seed to fail to germinate.
2. When a concentrated formula like 1-50-3000 is used, the amount of injury depends on the moisture condition of the soil. Injury varies inversely as the percentage of moisture.
3. The amount of injury may be reduced by diminishing the amount of the solution applied per unit of row, but the percentage of smut control is reduced in the same ratio.
4. The amount of injury in a dry soil may be reduced by increasing the dilution of the formaldehyde without at the same time reducing the actual amount of formaldehyde per unit of row.

5. The amount of injury can be predicted from the moisture condition of the soil on the day of planting. It is not affected by weather conditions during the subsequent days.

6. The grower could save time and labor by changing his formula according to the soil condition at the time of planting.

7. More seed should be applied when formaldehyde is used.

DEPARTMENT OF BOTANY,

MASS. AGR. EXP. STA.

SEVERITY OF ATTACKS OF BLACK STEM-RUST IN DENMARK, 1884-1921

HANS R. HANSEN

Many observations made in Denmark by growers for more than a century show that the interplay existing between black stem-rust (*Puccinia graminis*) and barberry is the cause of the widespread and destructive attacks of the former. Gradually as more and more evidence of the guilt of the barberry was produced, the campaign for and against the eradication of that plant waged hotter and hotter. Even after scientific tests had confirmed the evidence of the practical man, the fray continued and was fiercest among the farmers themselves.

In 1869 an attempt was made to exterminate the barberry by law, a paragraph in the Enclosure Act of March 6. However, it is certain that this paragraph was seldom put into effect, for barberry bushes continue to exist in great numbers and the black stem-rust to destroy. By untiring efforts for many years, Professor E. Rostrup makes clear the importance of exterminating the barberry. He collects evidence of the attacks of black stem-rust co-existing with the barberry bush in various sections of Denmark and publishes the facts in annual reports from 1884. During the nineties Rostrup estimates that the damage caused by stem-rust amounts to about 10 million kroner annually. At length he succeeds in promulgating a measure forbidding all cultivation of the barberry bush in Denmark outside of botanical gardens. On March 27, 1903, the measure becomes a law which goes into effect in 1904. At present the barberry bush may be said to have totally disappeared, even though a stray bush or two may be occasionally found in gardens. Simultaneously with the disappearance of the barberry bush the severe attacks of rust have also ceased.

The annual reports on plant diseases issued first in 1884 by Professor Rostrup, later continued by Professor Kolpin Ravn and now by the Phytopathological Experiment Station give a summary of the severity of the attacks of each year. According to these reports the attacks may be grouped into 3 classes: great damage in large sections of the country, sporadic appearance without importance, and very seldom found. In the second column of the table the severity of the attacks in the years 1884-1921 is shown.

TABLE 1—*Severity of attacks of black stem-rust and climatic conditions of the summer*

Year	Severity of attacks	Deviation from average precipitation in pCt	Deviation from average temperature C°	Deviation from average cloudiness (0—10)
1884.....	⊙	÷ 88	+0,9	÷1,7
1885.....	⊙	÷ 62	÷1,6	÷0,5
1886.....	⊙	÷ 90	÷1,6	÷0,7
1887.....	⊕	÷140	0,0	+1,2
1888.....	⊙	+120	÷4,2	+3,1
1889.....	⊕	+ 24	+2,8	÷1,4
1890.....	⊙	+ 91	÷2,6	+2,6
1891.....	⊙	+122	÷1,1	+1,7
1892 .. .	⊙	+ 39	÷2,9	+0,6
1893 .. .	⊙	÷ 42	+1,9	0,0
1894.....	⊕	+ 37	+0,3	+1,3
1895 .. .	⊕	+ 66	÷0,3	+1,9
1896 .. .	⊕	÷ 49	+3,4	+0,2
1897.....	⊕	+ 24	+3,4	+0,9
1898.....	⊙	+ 87	÷3,0	+2,2
1899.....	⊙	÷167	+1,7	÷1,9
1900.....	⊙	+ 25	+1,8	+0,1
1901.....	⊕	+ 10	+3,2	÷0,1
1902.....	⊙	+ 4	+5,0	+2,5
1903.....	⊙	+ 51	÷2,1	+2,6

Barberry Law passed 1903

1904.....	⊙	÷113	÷0,6	÷0,9
1905.....	⊙	+ 35	+2,7	+1,0
1906.....	⊙	÷ 40	+1,0	+0,4
1907.....	⊙	+ 84	÷5,3	+4,6
1908.....	⊙	÷ 9	+0,6	+0,6
1909.....	⊙	÷ 2	÷3,7	+4,0
1910.....	⊙	+100	+2,2	+0,8
1911.....	⊙	÷ 31	+2,4	÷1,4
1912 .. .	⊙	+ 90	+0,4	+2,4
1913 .. .	⊙	÷ 89	÷1,9	+0,3
1914.....	⊙	÷ 49	+4,4	÷0,6
1915.....	⊙	÷ 54	÷1,6	+2,5
1916.....	⊙	+ 70	÷3,0	+3,6
1917.....	⊙	÷ 13	+4,7	÷0,4
1918.....	⊙	÷ 19	÷1,9	+1,7
1919.....	⊙	÷ 16	÷3,3	+2,9
1920.....	⊙	÷ 34	÷0,4	+2,1
1921.....	⊙	÷ 87	÷0,8	÷0,6

⊕ Great damage in large sections of the country.

⊙ Sporadic appearance without importance.

⊙ Very seldom found.

The years 1889, '94, '95, '96, '97 and 1901 show many severe attacks while the intervening years show few, unimportant attacks, and the years subsequent to 1901 no severe attack whatsoever.

It is certain that the summer climatic conditions, (June, July and August), as well as the climatic conditions of March and April, which determine an early or late sowing and a consequent early or late maturity of the cereals, are important factors in the spread, germination and infection of the rust spores. Late sowing, copious precipitation, low temperature and much cloudiness during the summer cause late maturity and consequent good conditions for a severe attack. Table 1 shows the climatic conditions of June, July and August; the figures for the separate months are added. From this we see that years, during which there were severe attacks of *Puccinia graminis*, contain one or many points of similarity.

Precipitation: above normal in 1889, '94, '95, '97 and 1901.
below " " 1896.

Temperature: above normal in 1889, '94, '96, '97 and 1901.
below " " 1895.

Cloudiness: above normal in 1894, '95, '96 and '97.
below " " 1889 and 1901.

1889—Early sowing; drought in the early summer; the subsequent copious precipitation caused uneven ripening; very late harvest.

1894—Early sowing; late harvest due to the climatic conditions of the summer.

1895—Rather early sowing; harvest slightly later than normal.

1896—Late sowing; early harvest.

1897—Late sowing in unsuitable soil; harvest somewhat later than normal.

1901—Very late sowing; severe attacks of frit-fly (*Oscinis frit*); harvest at normal time.

Either early sowing, unfortunate climatic conditions during ripening and late harvest, or late sowing, often in unsuitable soil, unfortunate climatic conditions during the early part of the summer, but harvest at the normal time, afford good conditions for attacks of black stem-rust.

Conditions similar to those mentioned above characterized the years 1903, '05, '10, '12 and '16.

Precipitation: above normal in 1903, '05, '10, '12 and '16.

Temperature: above normal in 1905, '10 and '12.

below " " 1903 and '16.

Cloudiness: above normal in 1903, '05, '10, '12 and '16.

1903—Early sowing; unfavorable harvest weather, late harvest.

- 1905—Late sowing; very severe attack of frit-fly; copious precipitation in August; late harvest.
- 1910—Early sowing; severe drought in the early summer; harvest at normal time.
- 1912—Late sowing; copious precipitation; much of the crop lodged; late harvest.
- 1916—Early sowing; germination in unsuitable soil; cold damp summer; late harvest.

On the whole these 5 years seem to reveal similar conditions, for the attacks of black stem-rust, to those in the 6 years referred to above during which very severe attacks of the disease were noted. Nevertheless no severe attacks of black stem-rust were observed during the period 1902-'21. The only possible reason for this lies in the absence of sources of contagion, and a careful 38 years' search of the country shows only one main source—the barberry bush—now practically eradicated. The few stray bushes hidden away here and there are of no importance in this connection; even though, wherever found they should be rooted out without mercy.

It is impossible to reach these results by rational experiments in a country where we may safely say that every spot is infected with black stem-rust; however, if we may consider all of Denmark as a single experimental plot, we have sufficient proof that the eradication of the barberry bush is synonymous with exemption from the destructive attacks of black stem-rust.

LYNGBY, DENMARK.

ELIAS JUDAH DURAND

R. B. HARVEY

WITH PLATE XVIII

Dr. Elias J. Durand was born at Canadaigua, New York, March 20, 1870, and died at St. Paul, Minnesota, October 29, 1922. He received the degree of A. B. (1893) and of Doctor of Science (1895) from Cornell University. As Fellow, Assistant and Instructor in Botany he served his Alma Mater from 1893 to 1910. In the latter year he went to the University of Missouri, where he remained as Associate Professor of Botany until 1918. From 1918 to the time of his death he was Professor of Botany at the University of Minnesota.

While Dr. Durand's contributions to botanical science have covered a wide range of subjects, he is best known to his colleagues in botany, both in this country and abroad, for his work in the field of mycology and especially as an authority on the Discomycetes.

Dr. Durand's herbarium has been deposited in the mycological collections of Cornell University, Ithaca, N. Y. It contains 12,087 specimens of Discomysetes fully indexed and classified with indexed notes on all of the specimens. There are also in the collection 6000 microscopic slides prepared from specimens and arranged to facilitate comparison of microscopic characters of the herbarium specimens.

Dr. Durand's publications, while principally in the field of mycology, have included articles on the Bryophyta and a Laboratory Outline of General Botany which has run thru five editions.

In his death, botanical science has lost a faithful scientist, an inspiring teacher, and a kindly personality.

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ELIAS JUDAH DURAND

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DEPARTMENT OF BOTANY,

UNIVERSITY OF MINNESOTA

PHYTOPATHOLOGICAL NOTES

Plant disease survey of England and Wales: This series of publications was briefly mentioned over a year ago. (*Phytopathology* 11: 376, 1921). The report for the years 1920 and 1921 is just issued as Miscellaneous Publication No. 38, of the Ministry of Agriculture and Fisheries, 10, Whitehall Place, London.

The two years were as different in climate as any two seasons ever are, hence the report is of especial interest. Three hundred and ninety one diseases are listed with notes on occurrences, severity of attack, relation to weather, etc. Weather charts accompany the report. This is the fourth report to be issued, the series covering the years 1917 to 1921, inclusive.—PERLEY SPAULDING.

Personals. Dr. James R. Weir, who for the past ten years has been in charge of the field investigations of the Office of Forest Pathology, Bureau of Plant Industry, for District 1 of the U. S. Forest Service, (Montana, Idaho, Western Washington, Western Oregon, South Dakota, Minnesota, and Michigan) with headquarters at Missoula and Spokane, has given up this position and is now directing the work of Pathological Collections in the same Bureau. This Office deals with the classification of fungi and maintaining the extensive mycological collections of the U. S. Department of Agriculture. In the immediate future Dr. Weir will accompany the Bureau expedition to the headwaters of the Amazon and other South American regions, as pathologist in rubber investigations. The expedition sailed from New York in July.

Dr. H. H. York, Pathologist in the Office of Forest Pathology, Bureau of Plant Industry, U. S. Dept. Agr., has accepted the position of Forest Pathologist, Division of Forest and Lands, Conservation Commission, Albany, N. Y., beginning July 1, 1923. For the present he continues work upon the white pine blister rust but other forest disease work will be taken up later.

A special institute for phytopathology has been founded at the Landwirtschaftliche Hochschule Bonn-Poppelsdorf, and Dr. E. Schaffnit has been made professor in charge.

The August number of Phytopathology was issued September 8, 1923.

PHYTOPATHOLOGY

VOLUME XIII

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THE MELANCONIS DISEASE OF THE BUTTERNUT (*JUGLANS CINEREA* L.)¹

ARTHUR HARMOUNT GRAVES

WITH PLATES XIX AND XX AND 5 FIGURES IN THE TEXT

INTRODUCTION

For about twenty years the writer has been particularly interested in the general pathological condition of the butternut, (*Juglans cinerea* L.). During this period several trees in Connecticut of various ages, on which observations were made from year to year, have finally died, and others have been gradually growing weaker. (Pl. XIX, figs. 1, 2). Previous to a note by the writer, in 1919, (8) there was no mention in the literature of any serious trouble; although the leaf spot caused by *Gnomonia leptostyla* (Fr.) Ces. and De Not., (the perfect stage of *Marssonia Juglandis* (Lib.) Sacc.) has been reported to sometimes cause considerable damage (14, 19), and also the basidiomycetous wood-rotting fungi doubtless do not neglect this tree (14).

A thorough investigation of the cause of the trouble was begun in the summer of 1918, during a survey of diseases of trees in Greater New York. In this region, and in the same year in Pennsylvania and Maryland, the butternuts were in such a weakened condition as to compel attention. At this time the acervuli of an imperfect fungus, *Melanconium oblongum*, were found to be of quite general occurrence on the dead branches, and this organism was therefore tentatively assumed to be the cause of the trouble, particularly when greenhouse experiments with healthy twigs inoculated with the fungus in question gave positive results.

DISTRIBUTION AND ECONOMIC IMPORTANCE OF THE DISEASE

In many of the excellent "tree books" now current, one finds statements like the following:—

"Trees of the same age are apt to vary in vigor and size, dead branches are likely to appear early, and sound trees 8 or 10 inches in diameter are seldom seen;" (5) or,

¹ Brooklyn Botanic Garden Contributions, No. 31.

"While the butternut is often a very attractive tree as seen growing spontaneously, it is not to be depended upon for ornamental planting, as it is very apt to develop dead branches which interfere with its symmetry. Good-sized trees, even when growing under favorable conditions, generally show this disfigurement, and larger trees are very likely to die completely before they attain any great size." (7)¹

Dr. G. P. Clinton tells us that he has noticed the weakened condition of the butternut in Connecticut for a number of years, and calls attention to it in a recent bulletin (4), citing the work of the writer. Dr. L. O. Overholts, who recently collected the causal fungus in the mountains near Pennsylvania State College, writes that it "certainly has the appearance of being parasitic in this instance." Prof. J. Franklin Collins has noticed sickly trees in Rhode Island, and in 1922 sent us specimens of both perfect and imperfect stages of the fungus. Dr. C. H. Kauffman writes that he has made collections in New York and Maryland; and likewise Dr. A. S. Rhoads, in New York.

This testimony, taken in connection with the observations of the writer in Connecticut, New York, Pennsylvania, and Maryland, and with his study of exsiccati (p. 415) of the fungus in question, i. e. *Melanconium oblongum* Berk., and its perfect stage *Melanconis Juglandis* (E. & E.) comb. nov., on butternut, certainly justifies the conclusion that the disease is widespread, and very probably extends over the entire natural range of the butternut.

The disease is important *per se*, because it eventually brings about the death of the tree, although its progress is slow. During its course, more and more twigs and larger branches are killed, and this means, naturally, a progressive loss in the total photosynthetic activity. Typical, rather young trees, about 30 years of age, have been noted in the forest with approximately one third of all their large branches dead. Such a loss cannot fail to have a reactionary, weakening effect on the whole tree. But many trees have also been observed which are almost entirely dead while still young (10-20 years). Older trees with only a few living branches remaining are common. (Pl. XIX, fig. 2). In general, taking into account the shortening of the life of the tree as well as its slower growth due to this disease, we may perhaps estimate the loss of normal healthy growth of butternut timber to be somewhere between 30 and 50 per cent.

¹The following extract from a letter from Dr. Robert T. Morris, the well-known nut culturist, is of interest: "Over the greater part of Connecticut when I was a boy we had magnificent butternut trees, and on my country place at Stamford the last one of the majestic ones is now dead. Most of the younger generation died before they were thirty years of age, many of them at fifteen or twenty years."

We do not believe however, that there is danger of disappearance of the butternut, due to the activities of the fungus associated with this trouble, as has been claimed. (13) The disease ordinarily progresses so slowly that there is ample opportunity for reproduction of the tree and thus for perpetuation of the species.

But while the present paper is confined mainly to the disease in its relations to the butternut, the trouble is of vastly greater significance in its potentiality with regard to other species of *Juglans*. The fungus has been collected by the writer on *Juglans nigra*, one of our most valuable species of hard woods, and preliminary work (p. 431) has shown that other species of *Juglans* are susceptible.

From a larger, practical standpoint, the economic importance of the disease in the butternut is bound up with the economic value of the butternut tree itself.¹

Besides its timber, which is much esteemed for certain purposes, the tree has several well-known minor uses (13, 17), so that a disease which seriously threatens it should be regarded as of considerable economic importance.²

SYMPTOMS OF THE DISEASE

It should be emphasized first of all that the progress of the disease, in typical cases, is very slow. No sudden wilting of the leaves occurs—nor does any well marked lesion or canker, as in the chestnut blight, develop on infected branches.

From observations extending over a period of three years made on 62 trees in various localities in Connecticut and New York, the course of the disease is essentially as follows: small branches, either from the sides of the trunk, or from a larger branch, die slowly in basipetal succession, the disease to all outward appearances proceeding from the distal end of the branch toward the trunk. If this happens during the

¹ A botanist of some standing, in the course of a conversation with the writer about this disease, remarked that our country could well get along without the butternut. With the same line of reasoning one may argue that many of our native timber trees of secondary commercial importance may be dispensed with, and thus eliminate them, one by one. But we are losing all of our chestnut, a tree of the first magnitude; the white pine, the most valuable coniferous species in the Eastern United States, is seriously threatened; and because of the increasing scarcity of better woods many species formerly considered of minor importance, are coming into service, and have a constantly rising value. Certainly this policy of *laissez-faire* as voiced by our colleague, is greatly to be deplored and is diametrically opposed to the principle of conservation.

² Butternut is included in the list of 100 important eastern forest trees in "Important Forest Trees of the Eastern United States." U. S. Dept. of Agr. Dept. Cir. 223. 1922.

growing season, the leaves of the branches in question turn yellow gradually and drop off—in such slow succession, however, as to pass unnoticed by the casual observer—or the gradual death of the branch may occur in the leafless season. The bark of the dying branches changes from its natural dark greenish brown tinge to a reddish brown and finally a dull gray. Soon it becomes studded with small, dark, smooth papillae, which at length burst open exposing the black pustules or acervuli of the fungus, (Pl. XX, figs. 3, a, b, c). A year or so later all that remain of the pustules are small rounded or irregular holes in the loose outer bark (Pl. XX, fig. 7).

The disease is characterized by a succession of new, rather short branches which die in their turn, developing farther and farther down the main branches or leaders. Occasionally a crown of short lived suckers which soon become infected, appears on the trunk itself. In the last stages of the disease the tree is marked by the bare, gray, weathered wood of dead branches in the upper part, giving a characteristic stag-headed aspect (Pl. XIX, fig. 2). At this period, too, basal shoots often arise which soon become infected.

THE FUNGUS

Synonymy

In its imperfect form, the fungus associated with the disease has been known since Berkeley first described it from specimens from the United States in 1874 (2) as *Melanconium oblongum*. This is the stage which is usually found on diseased butternut and other species of *Juglans*. In 1893 Dr. Shear collected another form in close proximity to the conidial form on dead branches of butternut, and according to a note by him in the herbarium of the New York Botanical Garden, he believed it might be the perfect stage of *Melanconium oblongum*. In the same year Ellis and Everhart named this form *Diaporthe Juglandis* from Shear's material, citing his collection. Since the writer, by culture work (p. 420), has established beyond question that *Diaporthe Juglandis* E. & E. is the ascogenous stage of *Melanconium oblongum*, it seems advisable to assign the fungus to the genus *Melanconis* of the family *Melanconidaceae*, for this group is characterized by superficial conidia (except in *Valsaria*) of the type of *Melanconium*. *Melanconis carthusiana* Tul. was long ago (12) connected with *Melanconium juglandinum* Kze., which is of common occurrence on *Juglans regia* in Europe. This conidial form is quite similar to *Melanconium oblongum*, but a careful examination and comparison of exsiccata of the two forms has convinced us that they are

distinct. The following new combination is therefore proposed for this American species of *Melanconis*, and a description is here added to supplement the characterization of *Diaporthe Juglandis* by Ellis & Everhart. (6)

MELANCONIS JUGLANDIS (E. and E.) comb. nov.

Perithecia seated in scanty stroma $\frac{1}{8}$ to $\frac{1}{2}$ mm. below surface of bark, in groups of 1–9, usually not more than 5, sometimes 1, globose, or slightly flattened tangentially, black, 500 μ in diameter, (range $441 \times 617 \mu$) their necks black, about 90 μ in diameter, and ordinarily only slightly extruded; asci cylindrical or slightly club shaped, $108 \times 11.7 \mu$ (range $90\text{--}110 \times 10.5\text{--}12 \mu$), with a short stipe about 6 μ long (range 3–9 μ); ascospores hyaline, colorless to light grayish olive—(15, Pl. XLVI), elliptical, with blunt ends or sometimes pointed, 2-celled and slightly constricted at the septum, averaging $20 \times 8.5 \mu$.

Pycnidia developing in culture, black, spherical or irregular, partly sunk in the medium, 150–400 μ in diameter, with a rounded apical pore, and surrounded by a dense felt of mycelium. Pycnosporos oblong, hyaline, one-celled, $1 \times 3 \mu$, swelling markedly on germination.

The conidial form is *Melanconium oblongum* Berk. with usually inequilateral spores, averaging $19 \times 9 \mu$, olive gray (15, Pl. LI) under the microscope, but appearing jet black when massed in the acervulus.

Habitat. North America.¹ A slow parasite in wood and bark of *Juglans cinerea* L. and *J. Sieboldiana* L.; also occurring on *J. nigra* L. and *J. regia* L. and perhaps pathogenic there.

HOSTS AND LOCALITIES OF OCCURRENCE

In the following list, which makes no pretensions as to completeness, all specimens except those starred have been examined by the writer. On *Juglans cinerea*.

CANADA: London, May 13, 1892; no definite locality, February 11, 1893, "Macoun."

MAINE: North Windham, July 26, 1923, A. H. G.

RHODE ISLAND: Lincoln, September 30, 1922, (perfect stage) *J. Franklin Collins*.

CONNECTICUT: West Hartford, September 24, 1919, A. H. G.; Meriden, September 16, 1922; Newington, September 16, 1922; Berlin, September 16, 1922; Hamden, January, 1919; July 2, 1920; October 21, 1920 (perfect stage); September, 1921; January 3, 1922; September 16, 1922 (perfect

¹ In the list of Danish fungi in Dr. Rostrup's herbarium (11) one specimen of *Melanconium oblongum* is included with the statement that it occurred on *Juglans mandshurica* in the garden of an Agricultural High School and is new for Europe.

stage), all *A. H. G.*; North Branford, December 19, 1920, *A. H. G.*; Sharon, July 12, 1920, *A. H. G.*; Southport, September 27, 1922, *A. H. G.*; Norwalk, September 27, 1922, *A. H. G.*

NEW YORK: Farmington, July, 1889, *E. Brown*; Canton, 1857, (no collector given); also a second specimen from this same locality without date or collector; Buffalo, no date, *G. W. Chnton*;^{*} Alcove, August 1893, "with *Diaporthe juglandis* E. & E.," *C. L. Shear*; Potsdam, no date, (N. A. F. 121); Van Cortlandt Park, New York City, August 16, 1918, *A. H. G.*; Cold Spring Harbor, August 14, 1918, *A. H. G.*; Inwood, New York City, January 3, 1919, *A. H. G.*; Baldwin, L. I., December 6, 1921, *A. H. G.*

NEW JERSEY: Newfield, June 1892. (*Fungi Col.* 153).

PENNSYLVANIA: Kennett, September, 21, 1918, *A. H. G.*; Shingletown Gap, Center County, September 26, 1922, *L. O. Overholts*.

MARYLAND: Glen Echo, January 9, 1919, *C. H. Kauffman*.^{*}

WEST VIRGINIA: Belleville, December 27, 1921, *Lee Bonar*.^{*}

WISCONSIN: St. Croix Falls, November 1, 1897, *C. F. Baker*.

IOWA: No definite locality, May 1882, *E. W. Holway*.

On *Juglans nigra*.

NEW YORK: Valley Stream, L. I., April 1921, *A. H. G.*; Baldwin, L. I., December 16, 1921, *A. H. G.*

On *Juglans Sieboldiana*.

CONNECTICUT: Stamford, July 20, 1919, *A. H. G.*; New Haven, July 31, 1920, *A. H. G.*

NEW YORK: Ithaca, campus of Cornell University, March 28, 1904, *C. H. Kauffman*;^{*} Baldwin, L. I., December 16, 1921, *A. H. G.*; Brooklyn, (from inoculated seedlings), June 12, 1922, *A. H. G.*

On *Juglans regia*.

CONNECTICUT: Stamford, July 20, 1919. *A. H. G.*

NEW JERSEY: Newfield, 1892, *Ells* (N. A. F.).

MORPHOLOGY OF THE FUNGUS

Mycelium

In a culture two days old on nutrient medium, the hyphae are hyaline, colorless and copiously branched, the main trunks being from 6–8 μ in diameter (fig. 1, a, b). The cross walls are distinct, the hyphae being often slightly constricted at these points. These numerous branches, constrictions, and also frequent, short, knubbly branches give the young mycelium a characteristic ragged aspect. Various colors and color changes develop under the influence of different culture media, but in old

cultures the prevailing shades are olives and grays. Under the microscope, old mycelium grown in oat agar has a yellow-green shade. In old cultures the main hyphal trunks are of about the same diameter as in the young cultures, but there may be a host of many fine hairlike branches about 2μ in diameter with cross walls indistinct. Between these two extremes, all gradations exist. In connection with the development of conidia and pycnidia in cultures, a smoke gray, fluffy, aerial mycelium

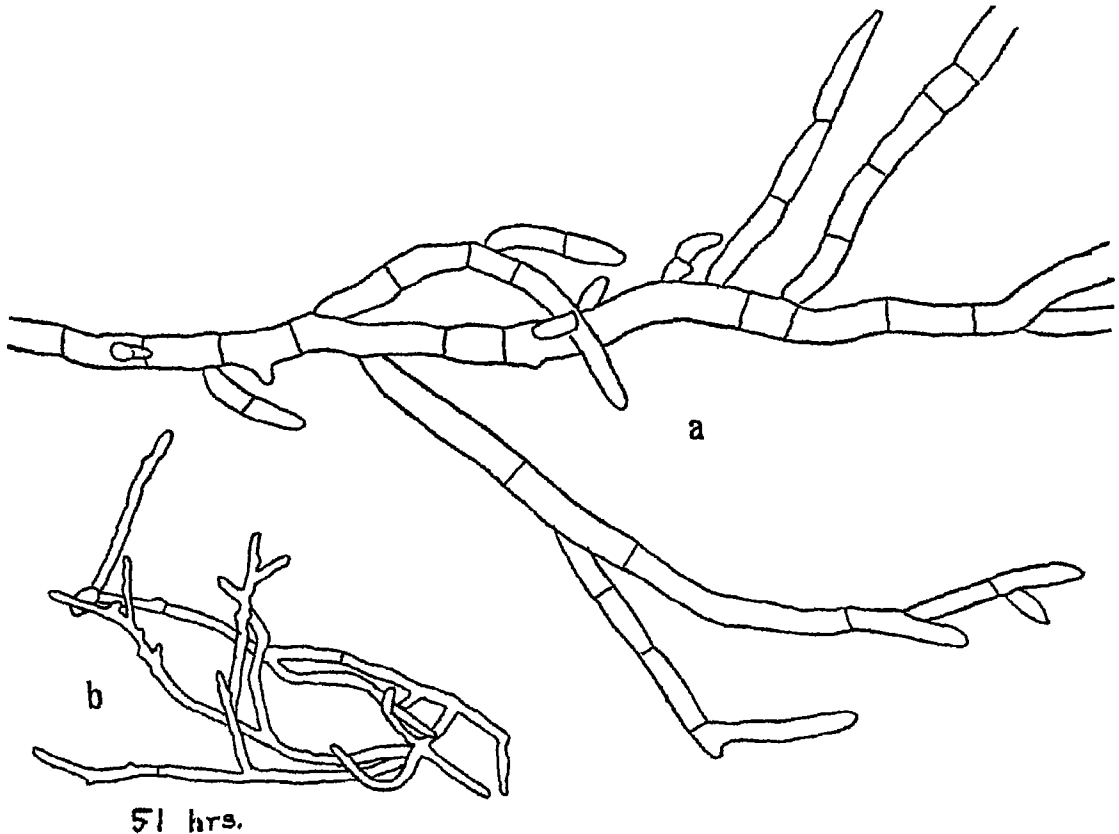


Fig. 1. Mycelium from a young culture: a, $2\frac{1}{2}$ days old, $\times 410$; b, 51 hours old, $\times 110$.

appears, often in cushionlike tufts. This is composed of very fine hyphae essentially similar to the fine branches in the medium. In the bark of the host, the mycelium is even more slender—from 1 to 1.5μ in diameter.

Conidial Stage

It is this form which is almost invariably seen on dead butternut branches, at first developing small, rounded, pimply, blackened elevations, scattered irregularly about (Pl. XX, fig. 3, a, b) and later bursting through the epidermis and young cork layers with rounded, irregular openings (Pl. XX, fig. 3, c) revealing the pustules of the apparently inky-black conidia. Although pustules appear usually only on the twigs

and small branches, occasionally they are found on the thick bark of the trunk (Pl. XX, fig. 4); and as shown by the figures (Pl. XX, figs. 5, 8, 9) there is much variation in their size. Sometimes they are elongated or run together (Pl. XX, fig. 9). If the weather is damp or rainy, they resemble tiny drops of thick ink, or if the twigs are placed in moist chambers, with the humidity of the correct percentage, the conidia are extruded in tendril form (Pl. XX, fig. 5). We have rarely seen the tendril form in nature, however. With age, the pustules become more flattened like small ink blots on the branch; and at length, after the lapse of a year or even less, are represented merely by rounded or irregular holes in the loose, outer bark, the spores having presumably been washed away or perhaps eaten by insects (Pl. XX, fig. 7). Saccardo (16) gives $20\ \mu$ for the length of the conidia. Our measurements average $19.1 \times 9.3\ \mu$ for 150 spores. The range of all the specimens examined was $14\text{--}25 \times 4.25\text{--}13\ \mu$. This, however, excludes a single giant spore found in the Iowa material, measuring $30 \times 13\ \mu$.

In shape, the conidia vary considerably; typically somewhat inequilateral, or with one side curving outward, in some specimens a kidney shape prevails, intermixed with perfectly oval and also symmetrically elliptical forms (Fig. 2, a); ordinarily muticate, sometimes they are bluntly pointed and occasionally terminate at one end in a narrow, short truncate base. Abnormally curved forms also occur, especially in cultures. The optical cross section shows usually a somewhat flattened outline, but it may be quite circular. Crystals of calcium oxalate, such as Johnston (9) records for *M. sacchari*, are common in the pustules.

In color, using daylight illumination, the contents seem to be an olive gray to a deep olive gray (15, Pl. 51) depending upon the amount of light admitted through the diaphragm, but with much darker brownish walls; in fact, it is the wall which gives the dark color to the spore as can be determined by crushing. The contents are then shown to be in reality much paler, though still a shade of gray. Spore specimens which have been kept in glycerine mounts for three years have retained their natural color well.

As to contents, the conidia are densely packed with light-refracting granules, and sometimes contain one droplet or several small ones (Fig. 2, b).

As indicated above, there is some variation in size in the material from different localities, that from Iowa being the largest, averaging $21.0 \times 11.0\ \mu$, but taking into consideration all the spore characters, the form seems quite distinct from other *Melanconiums*.

Pycnidial Stage

This was found only in cultures—in peptone agar in large numbers, and in oat agar (p. 421); in the latter case developing at about the same time as the conidia and surrounded by them. The pycnidia are black to the naked eye, spherical or irregular in shape, 150–400 μ in diameter, with

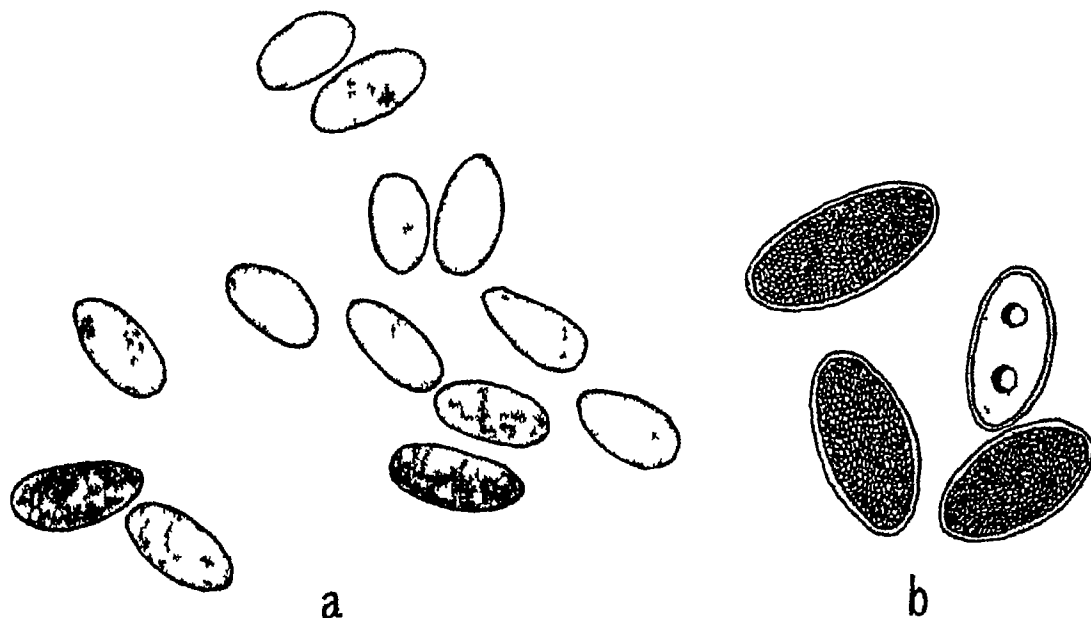


FIG 2 Conidia: a, showing different forms, $\times 600$: b, showing granular content or droplets, $\times 1000$.

a rounded apical pore (sometimes 2 pores if of irregular shape), and partly sunk in the medium, being surrounded by a dense felt of pale smoke-gray mycelium. When placed in water, enormous numbers of tiny, oblong, hyaline pycnospores, (Fig. 3, a) about $1 \times 3 \mu$, issue from the openings.

Ascogenous Stage

On Oct. 9, 1920, some of the dead twigs of a living butternut at Hamden, Conn., were observed to be not only plentifully covered with *Melanconium oblongum* pustules, but in and about these same pustules was what appeared to be the perfect stage in cracks in the bark (Pl. XX, fig. 10 and text fig. 4). The twigs were incubated in a moist chamber, and under these conditions, groups of long, black, hairlike structures, the necks of perithecia, grew out from the cracks (Pl. XX, fig. 11). Mature spores, shot out from the asci through these necklike openings,

were caught on slides suspended above, and sowings were made in oat agar on petri dishes under sterile conditions. These cultures matured typical conidia of *Melanconium oblongum* in 22 days. On December 2, the experiment was repeated, and on January 3, 1921, conidia again appeared.

The perithecia found at Hamden, Conn. were compared with the material at the New York Botanical Garden under the name of *Diaporthe Juglandis* E. & E. (p. 414) and found to be identical, with the exception, of course, of the long, extruded necks which had developed under cultural conditions.

On October 7, 1922, we received specimens labelled *Diaporthe Juglandis* E. & E. recently collected on butternut in Rhode Island by Prof. J. Franklin Collins. Examination showed that these also were the perfect stage. In October, 1922, the writer again collected the perfect stage at

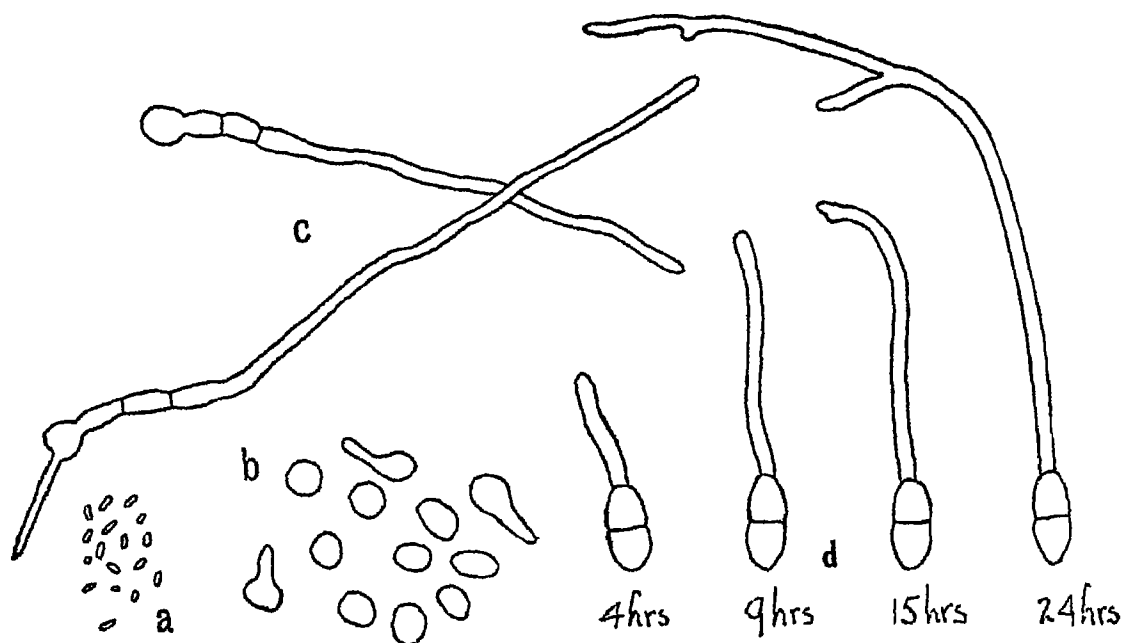


Fig. 3. Pycnospores; a, freshly ejected from pycnidia; b, 15 hrs. later, commencing germination; c, 18 hrs. after a, showing well developed germ tubes, $\times 500$; d, germinating ascospore, $\times 500$.

Hamden, Conn. It is extremely inconspicuous, being located in small cracks in the bark which when not as numerous as in the twig figured (Pl. XX, fig. 10), might pass for old lenticels.

Agar Cultures

Turnip juice agar, oat agar, potato juice agar, peptone agar, and butternut twig agar were all used as media, but for the production of

spores the oat again gave by far the best results. This was somewhat modified from Dr. Clinton's formula (See Rept. Conn. Agr. Expt. Sta. 1909 10 760ff) as follows: fifty grams of rolled oats were cooked in 300 cc. of water in a double boiler for about one half hour then pressed through cheesecloth or a fine wire strainer. 12 g. of agar added and enough water to bring the whole volume up to 1000 cc. After boiling in the autoclave to melt the agar titration showed the medium to be nearly neutral. After sterilization the medium was opaque and nearly smoke gray (15 Pl. XLVI) ¹

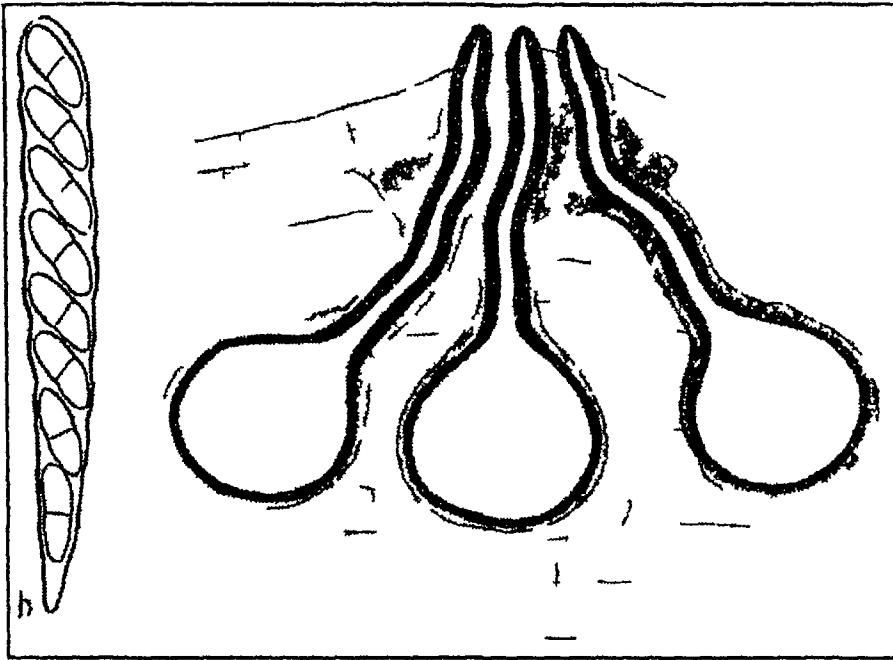


Fig. 4 a Diagram of perithecia in ball $\times 45$ b ascus with spores $\times 550$

Cultures were derived from a single spore and obtained by the isolation and transplantation method as follows. About two days after germination in the isolation petri dishes the tiny cultures could readily be distinguished by holding the petri dish up to the light, one selected which seemed remote from any impurity and then its origin from only one spore confirmed by examination of the petri dish directly on the stage of the microscope. Transplantation with a sterile scalpel to a dish of sterile medium was then a simple matter. Contamination in this process was inevitable but if a considerable number of transplants were made some would prove pure.

¹Roman numerals refer to plates in Ridgway's Color Standards and Nomenclature (15)

The fungus shows marked differences of behavior in the different media, but only its characteristics in the oat agar will be described in detail. The young mycelium (3 days old) is an ochraceous-buff (15, Pl. XV), which deepens (8 days) into an ochraceous-orange (15, Pl. XV), and at length (2 weeks) assumes shades varying from olive-ocher to yellowish olive (15, Pl. XXX) mottled with mummy brown (15, Pl. XV). Tisdale (21) notes the presence of green shades in his cultures of *Melanconium* from tomatoes. Later, the color becomes more uniformly dark, and aerial mycelium, which up to now has been scanty, develops sparsely. In about 3 weeks a curious metallic luster appears on the surface of the medium, the center of the culture becoming nearly black. Small fluffy mounds of a smoke gray (15, Pl. XLVI) mycelium develop, in which the apparently coal black masses of conidia appear, like drops of thick ink. These changes vary somewhat, both qualitatively and quantitatively, and in time intervals, in different cultures, depending perhaps on the thickness of the medium, amount of moisture, temperature, etc.; but the sequence of colors is fairly uniform. In turnip juice agar and in potato agar, the mycelium has a tendency to wrinkle the surface of the medium. It should be noted that in all the cultures the growth was extremely slow, as compared with forms like *Penicillium* and *Rhizopus*.

Cultures on Sterile Butternut Twigs

These were cut into sections a few inches long, placed with a little distilled water in test tubes, sterilized, and inoculated with the fungus in the hope that the ascospore stage would develop on them. In some twigs after a little more than three weeks, conidial pustules appeared in the ordinary way, as small black pustules distributed in the bark. In other cases, the bark was fairly well covered with small cushions of mycelium (1-2½ mm. in diameter) of the characteristic olive shades—varying from a light olive gray (15, Pl. LI) to a deep grayish olive (15, Pl. XLVI) when old (Pl. XX, fig. 12). In about six weeks small, inky-black drops of conidia began to appear in these. This difference in behavior of these two sets of twigs was due perhaps to different moisture conditions. No perithecia developed in either case.

Germination of Spores

Conidia germinate readily in oat, potato, or peptone agar in 10-12 hours at laboratory temperature (Fig. 1, b). In tap water the germination is slight—one or two tubes in each drop—and in distilled water only in rare cases can germ tubes be seen.

Pycnospores sown in oat agar commence to swell and eventually assume enormous proportions, compared to their original size. Beginning as very tiny spores $1 \times 3 \mu$, at the time when the germ tube appears they are about 6μ in diameter, having taken on a more rounded form somewhat resembling yeast plants. Their volume thus increases nearly 40 fold (Fig. 3, a, b). A similar pregermination swelling occurs in the pycnospores of *Endothia parasitica* (1, p. 8), where it amounts to more than 85 times the volume of the resting spore. Germ tubes were put forth from the *Melanconis* pycnospores in from 15 to 16 hours at laboratory temperature. (Fig. 3, c.)

Ripe ascospores caught by the method described above (p. 421) and sown in oat agar germinated readily in 12-13 hours at laboratory temperature (Fig. 3, d). Germination occurred also in distilled water and in tap water, but it was poor—less than 1 per cent. Mycelium grown in oat agar from these ascospores produced typical conidia of "*Melanconium oblongum*" in from 3 to 4 weeks.

PATHOGENICITY OF THE FUNGUS

Data Based on Field Work

1. The fungus is constantly associated with this disease in the field. Nearly all wild butternuts of twenty years or more are affected to a greater or less extent. Trees of this age which do not show the conidial pustules, or the marks left by them on dead twigs (Pl. XX, fig. 7), are few indeed. The writer has yet to see a tree more than twenty years of age, whose limbs are accessible, on which in some part, the pustules of the fungus or traces of them, do not appear.

2. The proximal limit of the dead area on twigs or branches has been marked in the field and watched from year to year. This method has demonstrated the gradual encroachment of the fungus on healthy tissue, and subsequent appearance thereon of conidial pustules.

3. Records of 31 trees in a forest at West Hartford, Conn., have been kept since 1919, or through four growing seasons. These records show every year an increased number of branches infected with *Melanconis*. In some cases where the trees are vigorous, the number of healthy branches developing more than offsets those diseased; in others, the reverse is the case, while in others the total leaf area remains about the same from year to year.

4. The case in which the number of branches dying from this disease each year exceeds the number of new shoots developing, is illustrated by a particular tree in Connecticut which has been under observation for the last 16 years, and because it is considered a typical example of the progress of the disease, will be described here in some detail. Plate XIX, figures 1 and 2, which are photographs taken August 6, 1920 and July 30, 1922, show the course of the disease for a two year period. It is evident that the foliage in the later photograph is much more sparse, and a careful examination of the two figures shows that this is due to the death of many of the small leafy branches seen in the earlier photograph. For example, A, a branch of recent origin, formed in a sucker-like way at the base of a limb which has been gradually dying from the tip downward, is entirely dead in 1922. Similar sucker-like branches at B, bearing foliage in 1920, are now dead. At C, where a limb divides into three parts, a conspicuous growth has taken place in the two years, with development of many small branches, but few remain alive, so that the sum total of the foliage in 1922 is less than in 1920. On all dead branches which were accessible the *Melanconis* pustules were seen. The soil and light conditions for this tree are exceptionally good, so that there is no evident "physiological" reason for its condition. The fungus gained entrance probably through some dead or weakened branch perhaps 20-25 years ago, and the host has been undergoing a slow death ever since. Obviously only a short lease of life remains.

Inoculations of Healthy Twig Cuttings

As reported briefly in the preliminary note on this disease (8) vigorous cuttings of butternut were obtained in March, 1919, from wild trees, brought into the greenhouse and placed in jars of water. Fifty-nine inoculations with *Melanconis* conidia were made on 19 cuttings. Three more were used for checks, 8 incisions in all being made on these. On May 1 there were 33 infections, 19 of which showed the *Melanconis* conidial pustules, while 26 of the inoculations had healed and were apparently uninfected. Checks remained uninfected. A similar series on black walnut showed negative results on May 1.

In a second experiment of like nature, using twigs of butternut, Japanese walnut, and black walnut, the results were inconclusive because most of the cuttings soon withered and died. However, the black

walnut unfolded buds and continued healthy for some time, and this experiment is noteworthy mainly for the fact that out of 28 inoculation on this species, 5 developed the conidial pustules of *Melanconis*.

Inoculations of Forest Trees

1. In the spring of 1919 the branches and trunk of a young tree of butternut, about 5 inches in diameter, breast-high, were inoculated at 10 different points with a pure conidia-bearing culture of *Melanconis*. No careful record of the results was kept, but the inoculated branches were observed at irregular intervals and were eventually found in many cases to be plentifully besprinkled with the *Melanconis* conidia. The inoculations in the trunk healed over, and therefore in August, 1922, the tree was cut down to determine whether there was any internal result from these trunk inoculations. The incisions where the spores had been placed were found to be covered with four rings of healthy wood, but it was evident from the appearance of a dark discoloration that the fungus *had advanced both up and down in the trunk along the line of the 1919 ring of wood*. To determine the extent of this advance the trunk was cut into lengthwise and transverse sections which were left lying on the ground. On returning to the spot two months later, large numbers of the conidial pustules of *Melanconis* were found in the bark of these sections. This behavior of the fungus in the wood is most important to an understanding of its relations with its host, and will be discussed more in detail later.

2. A more careful series of inoculation experiments was commenced in July, 1919, at Hamden, Connecticut. Fifteen trees of *Juglans cinerea* of various ages were inoculated with conidia and the results noted at three inspections in the same year—August, September, and November. Inspections were made also in March, July, and November, 1920; in July 1921; and in July, 1922; so that these experiments cover a period of three years. One hundred inoculations and 20 controls were made.

In table 1 only the inoculations with positive results are given. With 8 exceptions, all others were made on large branches and on trunks ranging from 2 to 11 cm. in diameter, and externally, at least, gave no evidence of infection. During the first month no pustules had appeared, although according to field notes, in No. 62 there was an indication of slight elevations in the bark. By the second month, 3 of the inoculations gave positive results; in the fourth month, 5; at the expiration of one year, 17; two years, 24; and three years, 36. In the spring of 1922, however, a forest fire weakened trees II and III, with the result that the

TABLE 1—*Results of inoculations with Melanconis Juglandis on Juglans cinerea.*

No. of tree	No. of inoculation	Part of tree inoculated	Diameter in cm. at point of inoculation	Time of appearance of spore pustules (shown by + sign)				
				2 mos.	4 mos.	1 yr.	2 yrs.	3 yrs.
II	6	branch	1.4			+	+	+
"	7	"	1.5					+
"	8	"	1.2				+	+
"	9	"	1.3					+
III	10	trunk	2.1					+
"	11	"	2.0					+
"	12	"	2.0					+
"	13	branch	1.0				+	+
"	14	trunk	1.6					+
"	15	branch	0.7					+
"	16	"	0.9					+
VI	39	"	1.8			+	+	+
VII	40	trunk	12.5			+	+	+
"	41	"	12.5			+	+	+
"	42	"	12.0			+	+	+
"	43	"	11.5			+	+	+
"	44	"	11.5			+	+	+
IX	55	branch	0.3				+	+
"	56	"	0.9			+	+	+
"	57	"	0.4			+	+	+
"	58	"	0.4			+	+	+
"	59	"	0.9			+	+	+
"	60	"	0.5			+	+	+
X	61	"	0.8		+	+	+	+
"	62	"	0.4	+	+	+	+	+
"	63	"	0.4	+	+	+	+	+
"	64	"	1.0					+
"	65	"	0.8					+
"	66	"	0.4					+
"	69	"	0.9					+
"	70	"	0.4				+	+
"	71	"	0.5				+	+
"	72	"	1.0	+	+	+	+	+
"	73	"	0.6		+	+	+	+
XI	74	"	0.9				+	+
"	76	"	0.8				+	+
Total infections showing spore pustules				3	5	17	24	36

fungus appeared on nearly every branch, and taking this into consideration, it is fairly accurate to place the amount of apparent infection at about $33\frac{1}{3}$ per cent.

Of the 20 controls, four were made on trees II and III on healthy branches which died in 1922 when these trees became entirely infected. This was also the case with one made on tree VII which died completely in 1920, as noted below. The other controls, except in cases where they were too near inoculations, remained uninfected.

Several peculiarities in the results of these inoculations are noteworthy. First of all, in most cases the living bark around the lesion healed promptly, giving the impression that the fungus was not a pathogen at all. By degrees, however, an inoculated twig would turn a reddish brown, and, if in the growing season, would drop its leaves gradually, the pustules finally appearing as small elevations and later breaking through the bark. In such cases, even the callus which had healed over the incisions became covered with acervuli (Pl. XX, fig. 6).

Secondly, in nearly every case except, for example, in tree VII, the spore pustules appeared on *small branches* not more than 2 cm. in diameter—in the majority of cases, 1 cm. or less.

Finally, if the host had already been weakened, the fungus grew rapidly, as in tree VII. Here the organism grew rapidly from the start in the thick, living bark of the trunk, behaving as does *Endothia parasitica* on chestnut, although no spore pustules could be distinguished for about a year. Tree VII was of fair dimensions, about 5 inches in diameter, breast-high, but growing in a dense forest where it had been suppressed and badly diseased at the top, so that it was in a weakened condition. By the end of four months one inoculation, for example, showed 30 cm. of fungous growth upward, 15 cm. downward, and 7 and 20 cm. from the sides of the point of inoculation. In the bark thus killed, spore pustules developed, (Pl. XX, fig. 4), the only case seen where pustules occurred in the thick rough bark of the trunk. The tree died entirely in 1920 directly from the girdling action of the fungus.

In the case of trees II and III, already mentioned as weakened by a forest fire in the spring of 1922, the conidia appeared on all the inoculated branches soon after the fire occurred. A discussion of these peculiarities will be postponed for the present (p. 431).

Inoculations of Seedlings

Although the inoculations on forest trees brought out several new features regarding the operations of the fungus, it was felt that as regards defining its responsibility in the causation of the disease they lacked

conclusiveness. Among other objections which might be brought forward was the fact that in all of the larger trees selected *Melanconis* already existed on one or more of the branches, although remote from the inoculations. For it had been found practically impossible to locate trees of any size in the forest which did not show the fungus in some portion.

In order to obtain a more clear cut demonstration it was decided therefore to inoculate seedlings that were entirely free from the fungus, and a new set of experiments was commenced on these in December, 1921, in the conservatories of the Brooklyn Botanic Garden. Through the kindness of Mr. Willard Bixby, the well-known Long Island nut-grower and horticulturist, 45 two or three year old *Juglans* seedlings were secured, distributed about equally among the four species, *J. regia*, *J. nigra*, *J. cinerea*, and *J. Sieboldiana*. These young trees were potted in one-half loam and one-half leaf mold and placed first in a cool greenhouse, and later in one of moderately warm temperature. Under the influence of the latter, by March 1, 1922 all were in full leaf and growing vigorously.

One thing brought out in clear relief by the previous experiments was the indication that *Melanconis Juglandis* is a weak parasite, attacking rapidly only when its host is weakened. It was therefore decided to weaken some of the seedlings of each species, previous to inoculation, in various ways. Thus, two or three of each species, with their roots carefully washed and uninjured, were transferred to pots of clean washed sand; these and all others, with the exception of a first group (Table 2, Group 1) which were treated to optimum conditions of soil, water and light, were placed beneath the greenhouse bench where the light was poor, and watered sparingly. In addition, those in sand were pruned repeatedly beginning March 1, and continuing up to the time of inoculation (Table 2, Group IV). Another group (Table 2, III) was similarly treated except that they remained in good soil. Group II was similar to III except that there was no pruning. No seedlings of *J. nigra* were placed in Group II. In this way four lots of seedlings within each species (with the exception just noted) were developed under the conditions as stated in the first column to the left in table 2.

With one tree for a control in each of the sixteen groups, inoculations with *Melanconis* conidia were made on May 17, 1922. By June 12 one tree of *J. Sieboldiana* in class II was dead and well spotted with *Melanconis* pustules (Fig. 5). Table 2 gives the results to Mar. 1, 1923. Plate XX, figs. 8 and 13, from a seedling of *J. Sieboldiana*, show the progress of the disease from one inoculation down the stem to the junction of the

TABLE 2—Summarized results of inoculations (May 17, 1922) with *Melanconis Juglandis* on *Juglans* seedlings.

Physical conditions	Species	No. of seedlings	No. of inoc.	No. of seedlings showing development of spore pustules				
				Aug. 5	Sept. 1	Oct. 1	Nov. 15	Mar. 1
I Optimum.	<i>J. regia</i>	2	3					
	<i>J. nigra</i>	2	3					
	<i>J. cinerea</i>	3	4					
	<i>J. Sieboldiana</i>	2	2					
II Good soil, poor light, little water.	<i>J. regia</i>	2	2					
	<i>J. cinerea</i>	3	3			1	1	1
	<i>J. Sieboldiana</i>	2	2	2	2	2	2	2
III Good soil, poor light, little water, pruned.	<i>J. regia</i>	2	2					
	<i>J. nigra</i>	3	4		1	1	1	1
	<i>J. cinerea</i>	1	1					1
	<i>J. Sieboldiana</i>	2	2	1	1	1	1	1
IV Sand, poor light, little water, pruned.	<i>J. regia</i>	2	2					
	<i>J. nigra</i>	3	3					
	<i>J. cinerea</i>	3	4					
	<i>J. Sieboldiana</i>	1	2			1	1	1
Total		33	39	3	4	6	6	7

branch. The inoculation on the latter has apparently had no effect, but it is only a question of time when the fungus will descend below the origin of this branch and thus cut it off. None of the controls developed the disease, though one tree of *J. Sieboldiana* in class IV, in which the conditions are severe, has died. Although the results are not striking, they point clearly to the conclusion that the fungus is a weak parasite and that its attacks are most marked in a weakened host.

Leaving out the inoculations on healthy seedlings it will be seen that the amount of infection on the weakened seedlings is somewhat over 25 per cent. Incidentally it may be noted that the experiment brings out a marked susceptibility in *J. Sieboldiana*. Just why those in the sand have resisted the fungus so well is not clear. However, the fungus works slowly, as shown in the previous experiment (p. 426).

DISCUSSION OF THE RELATIONS OF FUNGUS AND HOST

The fact that in most of the inoculations on forest trees the incision apparently healed over, led the writer to believe at first that the fungus was not a pathogen, or at least only a very weak one; for one had to admit that it "took" very readily in the living bark of tree VII (p. 427). The comparatively sudden death of whole twigs after two months, followed by an increasing number of dying inoculated twigs and small branches each year up to the termination of the experiments, led very early in the work to a field investigation of the inoculations. It was

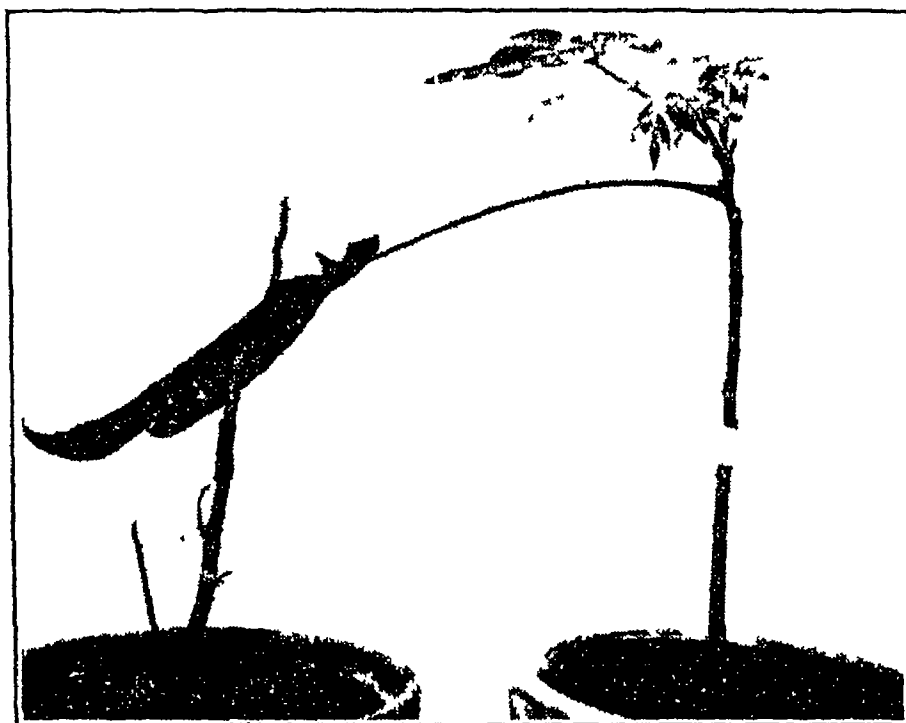


Fig. 5. Showing results of inoculation of seedling of *Juglans Sieboldiana* grown in good soil, but weakened by poor light and little water. Check plant at right. Spore pustules show on stem of inoculated plant at left. $\times$ about $\frac{1}{2}$.

found that under the callus a small, black area extended radially into the wood and thence had proceeded up and down the twig or branch a distance depending on the length of incubation. In both inoculated branches and twigs diseased from natural infections this black column, overlaid by healthy wood and bark, has been followed well into the trunk. In a small twig, it is conceivable that this fungal invasion, necessarily hindering the upward flow of liquids in the twig, would eventually result in so weakening it that the fungus will readily advance outward to cambium and bark—hence the death of the twig and sub-

sequent appearance of spore pustules. It is of course possible that the killing is due to the presence of some toxic substance secreted by the fungus or formed by the interaction of host and parasite, as Clayton (3) believes is operative in the tomato wilt. In the case of the larger twigs, naturally a longer time would be required for the lethal action of the fungus, whatever its *modus operandi*. In the trunk, in the case of a vigorous tree, the formation of the annual rings of wood goes on so rapidly as to cover up for a time the action of the fungus. Yet it may eventually reach a spot where small branches are given off, and advancing into these, slowly kills them. If the tree is weakened by drought or other unfavorable conditions such as fire (p. 427) the fungus advances more rapidly.

Sections of discolored wood have been stained, and show the presence of the fungous hyphae. The isolation of the fungus in culture media from such discolored wood has been extremely difficult and successful only in a few cases. Where segments of twigs with the discolored wood have been incubated in a moist chamber, they have invariably developed spore pustules. The case has already been described (p. 425) of the tree trunk inoculated in 1919, and cut in 1922, which showed the discolored wood covered by four layers of healthy wood, and developed the spore pustules after it had lain two months on the ground.

From the evidence presented in the foregoing it seems clear that we are dealing here with a weak parasite working slowly, or almost dormant, in vigorous trees—more rapidly in weak ones. After the lapse of many years it may gain the upper hand (Pl. XIX, figs. 1, 2) and the death of the tree is the result.

RELATIVE SUSCEPTIBILITY OF SPECIES OF JUGLANS

It will be seen by reference to the list of exsiccati and hosts that *Melanconis Juglandis* is by no means restricted to the butternut. Very probably the fungus may be found on any species of Juglans. But as far as our field observations go, pathogenic effects are most pronounced in *J. cinerea* and *J. Sieboldiana*, which is to be expected on account of the close relationship of those two species. In the experiments with healthy seedlings (p. 427), *J. Sieboldiana* proved extremely susceptible.

Although we have seen some trees of *J. nigra*, in the field, apparently seriously affected and showing all the symptoms of the trouble, we do not feel warranted at present, in view of the slight experience we have had with this species, in making any definite statement as regards its degree of susceptibility. But on account of its great economic importance

a thorough investigation is much needed. Likewise, regarding the susceptibility of *J. regia*, we know little.

As regards the western species of *Juglans*, we find no reference to a trouble like this. Thornber (20) states that the Arizona walnut, *Juglans major* Hell. does not appear to be troubled with any serious fungous disease. The walnut blight, so destructive to the Persian walnut, (*J. regia*) and its hybrids in California, is an entirely different disease. (18)

REMEDIAL MEASURES AND CONTROL

In the forest, any remedial or control measures are under present conditions impracticable. In view of the nature of this disease, as brought out by these studies, it is clear that trees with a large percentage of branches killed by *Melanconis* are in all likelihood trunk-infected. Their growth increment will necessarily be slow, the amount depending on the extent of infection. Such trees may as well be removed whenever practicable, as in improvement thinnings.

But in the case of orchard trees, or in parks and gardens, with proper care and vigilance the disease may be successfully combatted. In view of the fact that weakened trees in particular are attacked by the fungus, efforts should be made to maintain them in as healthy a condition as possible, by such sanitary measures as fertilizing, mulching, watering in dry seasons, pruning to increase vigor; and by avoidance of conditions which lead to winter injury, etc. The trees should be constantly watched, and all dead twigs pruned off as soon as discovered. It is hardly necessary to add that any twigs or branches showing fruiting bodies of *Melanconis* should be pruned off immediately, the cut being made some distance below the limit of the wood discoloration in the interior. All pruning wounds should be covered with paint or tar. After the fungus has penetrated the trunk, no remedy is practicable.¹

SUMMARY

The evidence indicates that the *Melanconis* disease extends over the entire natural range of the butternut, and is one of its chief troubles; while the cultivated Japanese walnut (*J. Sieboldiana*) is likewise particularly susceptible. The disease is also of significance in its possible relations to other species of *Juglans*.

¹ Dr. Robert T. Morris states in correspondence, that "if blighted butternut trees are cut near the ground, and the stump sprouts grafted later with Persian walnut, black walnut, or heart nut scions, we are likely to obtain good crops from this species which are highly resistant to attack from the *Melanconium*."

In trees which are previously weakened, the fungus makes rapid progress; otherwise the disease advances slowly. The evidence at hand does not appear to warrant the statement that there is danger of disappearance of the butternut.

In its first stages, the trouble is characterized by the appearance of dead limbs besprinkled with small black conidial acervuli. Occasionally these develop spore horns, but they are usually rounded or wartlike, and in wet weather like drops of thick ink.

This conidial stage has passed under the name of *Melanconium oblongum* Berk. The ascospore stage, which may appear later near these same pustules, has long been known as *Diaporthe Juglandis* E. & E. By culture work this is now definitely proved to be the perfect stage of *Melanconium oblongum* Berk. *Melanconis Juglandis* (E. & E.) comb. nov. is therefore proposed as the new name.

A pycnospore stage has appeared in cultures on both oat agar and peptone agar.

Inoculation experiments, extending over a period of more than four years, have demonstrated conclusively that the fungus is a weak parasite. Entering usually through small twigs by way of a wound, or through twigs already killed from some other cause, the mycelium grows slowly down through the wood, producing a black or dark discoloration, to the main branch and finally to the trunk. Thence any branch may become infected.

Ordinarily the progress of the disease is so slow that the leaves fall one by one, not producing any striking wilting or blighting effects. In final stages trees have a marked stagheaded aspect.

Diseased branches should be pruned off promptly some distance below the apparent infection, and the wounds tarred over or painted. After the fungus has penetrated the trunk, combative measures are useless.

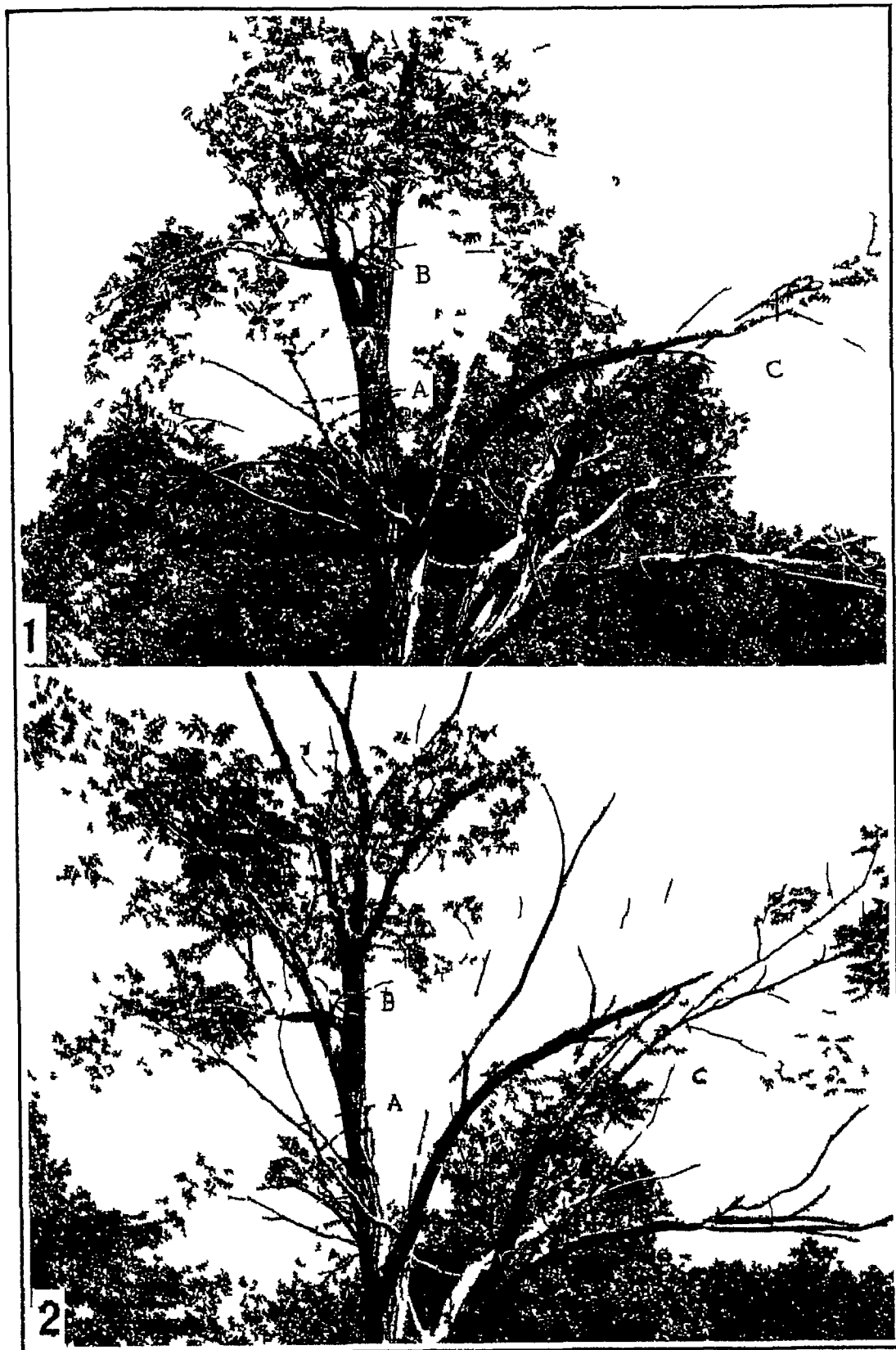
For forest trees, no remedy is practicable under present conditions. Diseased trees should be removed during improvement thinnings.

The writer wishes to acknowledge his indebtedness to the many who have contributed assistance and advice in this work—particularly to the following: Mr. A. B. Seymour, Harvard University, Dr. Robert T. Morris, New York City; Rev. Sidney Wallace, Waterbury, Conn.; Dr. G. P. Clinton, Mr. E. M. Stoddard, and Mr. Walter O. Filley, of the Connecticut Agricultural Experiment Station; Mrs. Flora W. Patterson, Bureau of Plant Industry, Washington, D. C.; to Professor A. W. Evans of Yale University, and to the staff of the New York Botanical Garden for placing at his disposal the facilities of their respective institutions;

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MELANCONIS JUGLANDIS ON BUTTERNUT



MELANCONIS JUGLANDIS ON BUTTERNUT

DESCRIPTION OF PLATES

PLATE XIX. MELANCONIS JUGLANDIS ON JUGLANS CINEREA

Figs. 1 and 2. A butternut tree at Hamden, Conn., showing the damage caused by the *Melanconis* disease in a two-year period. (1) Taken August 6, 1920; (2) Taken July 30, 1922. At A, B, and C are examples of branches which have died in the two-year interval. For detailed account see p. 424 of the text.

PLATE XX. MELANCONIS JUGLANDIS

Fig. 3. a, b, and c, successive stages in the development of the acervuli of the conidial stage of *Melanconis Juglandis* (E. & E.) comb. nov. on twigs of butternut: a, papillate swellings appearing; b, some of them burst open; c, warty, black masses of conidia. $\frac{4}{5}$ natural size.

Fig. 4. Acervuli, (the large black spots) on thick bark of trunk. $\frac{4}{5}$ natural size.

Fig. 5. Infected twig, incubated in a moist chamber, showing the extrusion of spores in horn-like masses from the acervuli. Such spore horns rarely occur in nature. $\frac{4}{5}$ natural size.

Fig. 6. Inoculated twig, showing where the inoculation was well healed over with a callus (about one inch from the base of photo); later, whole twig died as a result of growth of mycelium in interior, and numerous spore pustules appeared. This particular twig was a young green shoot at the time of inoculation. $\frac{4}{5}$ natural size.

Fig. 7. Part of old diseased twig showing holes in loose bark where spore pustules were located. $\frac{4}{5}$ natural size.

Fig. 8. Part of inoculated upright shoot of tree shown in Fig. 13, showing spore pustules in detail. Extent to which disease has apparently progressed is shown by irregular line below crotch. In the wood, however, the fungus has penetrated to the base of the tree, as was shown by subsequent examination. $\times 1$.

Fig. 9. Branch of *Juglans Sieboldiana*, showing spore pustules appearing about the point of inoculation, some of them elongated or run together. About natural size.

Fig. 10. Ascogenous stage (not quite mature) appearing in cracks of butternut twig. $\times 1\frac{1}{2}$.

Fig. 11. Showing perithecial "necks" excessively developed, under moist chamber conditions. $\frac{4}{5}$ natural size.

Fig. 12. Pure culture on sterilized butternut twig, showing mycelial mounds where conidia will appear. About natural size.

Fig. 13. Seedling of *Juglans nigra* showing two inoculations, one of which has "taken," the fungus having progressed downwards. For further details see description of Fig. 8. About natural size.

THE PROBLEM OF BREEDING NEMATODE-RESISTANT PLANTS

WALTER SCOTT MALLOCH

WITH TWO FIGURES IN THE TEXT

The serious nature of nematode infestation has been pointed out by numerous writers. *Heterodera radicicola* is the most destructive form known at the present time. The theme of the present paper is to present some data collected in an attempt to breed plants resistant to *Heterodera radicicola*. The control of the root-knot nematode under greenhouse conditions is relatively simple compared to field conditions. E. A. Bessey (3) has reviewed the different methods for the control of the root-knot nematode under greenhouse and under field conditions. Although nematode infestation is serious enough under any condition, I shall take up the discussion of its control from the viewpoint of field conditions, which is by far the most difficult problem. A serious nematode infestation of a highly susceptible perennial plant is generally fatal. About the only treatment which can be safely given is to apply fertilizers and constant cultivation to induce the trees to grow faster than nematode galls can be produced. This method, however, cannot be regarded as highly satisfactory, due to its high cost and temporary nature. Perennial plants are stationary, and once the soil becomes infested, the plants are doomed, sooner or later. Even if the soil is free from root-knot nematodes at the time the trees are planted, no one can tell when root-knot nematodes will spread to this piece of land.

W. A. Orton (7) has stated that, "The root-knot nematode is already so widely distributed in California that it will be extremely difficult to restrict its further spread in any effective way by the application of quarantine measures." The same statement would apply to many other states in the Union. With perennial plants, crop rotation cannot be applied without great financial loss. One may say, then, that the breeding of nematode-resistant rootstocks for fruit trees is by far the most important of nematode-resistant problems. What little has been accomplished in this direction by the writer has been submitted for publication in another article.¹

¹ Malloch, W.S. Asexual propagation as an aid to the breeding of rootstocks. (Submitted for publication.)

Let us now consider the problem of breeding for nematode resistance from the standpoint of annual plants. In breeding for disease resistance, the plant breeder has two alternatives: First, to find natural resistance in some variety or species and then to combine disease resistance or immunity with other desirable commercial characters. This is the most hopeful method from the breeder's standpoint. Second, it would be theoretically possible to create disease-resistant plants by transgressive segregation. This would imply that each parent possessed modifying factors which acting together would increase the resistance of the hybrid plants. Transgressive segregation does occur for certain characters such as tetramerous flowers in tobacco. Some varieties of tobacco have a reduced number of parts of one or more floral whorls. *Nicotiana Tabacum* var. *Havanensis* was found to produce on the average 18.814 ± 0.203 per cent tetramerous or otherwise reduced flowers on the same plant with the normal pentamerous flowers.¹ The extremes for this variety being 14 to 26 per cent. *Nicotiana Tabacum* var. *purpurea* on the other hand does not produce any tetramerous or otherwise reduced flowers. The F_1 hybrid between these two parents produced, on the average, 1.127 ± 0.792 per cent tetramerous or otherwise reduced flowers per plant. The F_2 produced all the way from 0.0 to 54.0 per cent tetramerous or otherwise reduced flowers per plant, thus exceeding either parent in the percentage of reduced flowers on any particular plant. In this case, the factor or factors for tetramerous flowers was clearly present in one parent. If a certain amount of resistance could be demonstrated in a particular variety, then we might hope to increase this resistance by hybridization.

Theoretically, the finding of resistance in one parent should not limit the building up of resistant forms. In the inheritance of aleurone color in corn, East and Hayes (5) found that two white varieties might each possess factors all of which are necessary for the production of color. A white variety of the genetic constitution CCrrppii crossed with another white variety carrying the factors ccRRppii gives a red type in F_1 and 9 red to 7 white in F_2 . I am not aware of any demonstrated case of this type in the inheritance of disease resistance. As a matter of fact, the reverse has been demonstrated. O. S. Aamodt (1) found in a cross between Kanred and Marquis wheats that all the F_2 plants were susceptible to biologic forms of *Puccinia graminis tritici*, to which both parents were susceptible. The controlled tomato hybrids made by the writer were all susceptible to nematode attack.

¹ Malloch, W. S. Studies of inheritance in tobacco and maize. Master's Thesis. 1920. (Manuscript.) University of California Library, Berkeley, California.

In breeding for disease resistance, it is frequently found that resistance is only obtained by crossing with resistant species. Beach and Maney (2) found that *Prunus besseyi* (Sand cherry) is immune to aphid attack, while *Prunus cerasus* (Montmorency cherry) and *Prunus Americana* (Wyant plum) are susceptible to aphid attack. These species cross freely and give a ratio of about 3 immune to 1 susceptible form in F_2 . But there are many species, like *Hordeum vulgare* and *Hordeum murinum*, which do not cross easily and in which the resulting hybrids do not reach maturity (6). In such cases as these, the knowledge gained by geneticists should be of great service to plant breeders.

EXPERIMENTAL RESULTS

In the description of the results of the present experiment, I have included a brief discussion of cultural methods for growing plants and nematodes together. This portion has been contributed for what little value it might be to further studies on the causes of immunity. A few more observations have been made on cross inoculation tests which serve to confirm the opinion that there are no physiological or biological strains of *Heterodera radicicola*. This is a distinct advantage to the breeding of nematode-resistant plants, for once a resistant plant is found, it should be resistant to any new infestation of *H. radicicola*. Such a condition is a disadvantage in crop rotation, however, as the same form of *H. radicicola* will attack many of the crops which would be desirable for rotation purposes. Finally, I have included a list of the species and varieties of plants found to be susceptible to *H. radicicola*.

CULTURAL METHODS OF GROWING PLANTS FOR STUDYING IMMUNITY TO NEMATODES

In order to study immunity of different varieties and species to nematodes and the causes for any immunity found, we must have some method of growing the plants and nematodes together. The natural method of infection from nematodes is from larvae present in the soil. Soil cultures are the most desirable for studying the relative resistance of different plants, but are unsuited for studying certain suspected causes of immunity, such as differences in anatomical structure. In the present investigation, a greenhouse soil badly infected with nematodes from Gardenia plants was used in the greenhouse tests. It was planned to test relative degrees of resistance by growing plants in the same type of soil which had been hauled to an isolated piece of land in Berkeley, but it was necessary to terminate the experiment before this could be accomplished.

For anatomical work, it is desirable to grow the plants in a culture medium, where the roots will be free from adhering soil particles. This may be accomplished by allowing the roots to grow through the drainage hole in a flower pot in which the plant is growing. It may also be accomplished by growing the plant in a nutrient solution. For chemical studies, it is often desirable to be able to control the culture media in which the plants are grown.

L. B. Byars (4) found that plants grown in Pfeffer's nutrient agar became infected when nematodes were added to the culture.

Pfeffer's Nutrient Agar

Calcium nitrate.....	CaNO ₃	4.0 grams.
Potassium nitrate.....	KNO ₃	1.0 "
Magnesium sulphate.....	MgSO ₄	1.0 "
Potassium dihydrogen phosphate.....	KH ₂ PO ₄	1.0 "
Potassium chloride.....	KCl	0.5 "
Distilled water.....	H ₂ O	6.0 liters.
Powdered agar.....		12.0 grams.

The writer found that Dwarf Champion (No. 13), Earliana (No. 73), and Matchum (No. 84) tomatoes and Whippoorwill (No. 2) cowpea became infected when nematode galls were added to the culture. The agar method has an objection in that it soon becomes infected with other organisms as observed by Byars (4), and Steiner and Heinly (8). The writer found that the agar became infected even when nematodes were not added to the culture media. Infection probably takes place when fresh agar is added to the bottle in which the plants are growing. In the present experiment, the details as to sterilization and preparation of the nutrient agar described by Byars (4) were followed out. At first the bottles were only filled one-third full with Pfeffer's nutrient agar. Sterilized seeds were sown upon the nutrient agar and the cotton plugs replaced. Most of the seedlings had a tendency to sink and become immersed in the fluid. Those which did not have this difficulty failed to grow more than about three inches, due probably to lack of air (Fig. 1).

To avoid this difficulty, tests were conducted with cowpeas which were sown upon the top of supports in the mouths of bottles. The bottle was filled with Pfeffer's nutrient agar until the sterilized seeds were partially immersed in the fluid. Two types of supports were used. An inverted strip of wire screening, one inch wide and six inches long, served for one type. Sterilized seeds were sown upon this wire support. The mouth of the bottle was then stoppered with a cotton plug. When the plants had germinated, the cotton was placed around the stem in such a manner as not to interfere with its growth above the bottle. A

piece of cheesecloth thinly covered with cotton and pressed down into the mouth of the bottle served as a second type of mechanical support. Sterilized cowpea seed was sown upon this support, which was in contact with the agar medium. In both methods, the plants developed normally to a height of about one foot (Fig. 2). Infection also developed in this method.

To avoid contamination from other organisms, an attempt was made to devise media in which both nematodes and plants could develop but in which other organisms could not. Nematodes were added to pure silica sand in a one-half pint milk bottle. Seeds from different plants were then sown in this bottle and the plants kept alive by frequent



FIG 1. Cantaloupe seedlings growing on Pfeffer's nutrient agar. The seedling in the bottle on the right has become immersed in the fluid.

additions of Pfeffer's nutrient solution. This solution has the same composition as Pfeffer's nutrient agar minus the agar. Whippoorwill cowpea (No. 2) and Red Currant tomato (No. 63) became infected in this culture medium. Such a method might be of value for certain chemical investigations but would be little better than a soil culture for anatomical studies.

Whippoorwill cowpea (No. 2) plants, five inches high, and Orobia (No. 76), Red Cherry (No. 81), and Striped (No. 78) tomato plants, one to one and a half feet high, which had been previously grown in nematode-free soil, were transplanted into Erlenmeyer flasks. Pfeffer's nutrient solution was maintained at about one-half inch depth in the flasks in which the plants were placed. The bottom of the flask was

about six inches wide, giving plenty of room for root development. The plants were grown in this way from Nov. 1, 1922, to Jan. 18, 1923. All of the varieties became infected and showed the presence of numerous galls on the roots. Algae developed both in the sand cultures and in the water cultures. The growth of algae may be suppressed, however, by keeping the culture media in the dark and only exposing the tops of the plants to the light. The water-culture method should be well suited for anatomical studies.



FIG. 2. Cowpea seedling growing on Pfeffer's nutrient agar. The one on the left is supported by wire screening. The one on the right is supported by a cheesecloth-and-cotton support.

CROSS-INOCULATION EXPERIMENTS

E. A. Bessey (3) has discussed the possibility of *Heterodera radicicola* developing physiological strains which prefer certain hosts and exhibit a reluctance to attack other hosts. Such strains are known in *Tylenchus dipsaci* and other organisms, and these strains are morphologically indistinguishable from one another. Carefully washed roots of a plant infected with *Heterodera radicicola* were placed in a pot of sterilized soil. Seeds of another kind of plant were sown in this pot, to determine if they became infected with nematodes of the other plant. Experiments

made in this way showed that *Heterodera radicum* were mutually interchangeable in the following plants: Red clover (*Trifolium pratense*), white clover (*T. repens*), crimson clover (*T. incarnatum*), cowpea (*Vigna unguiculata*), strawberry (*Fragaria chiloensis*), tree morning-glory (*Ipomoea syriaca*), sunflower (*Helianthus debilis*), horse bean (*Vicia faba*), ginseng (*Panax quinquefolium*), purslane (*Portulaca oleracea*), fig (*Ficus carica*), papaya (*Carica papaya*), catalpa (*Catalpa speciosa*), tomato (*Lycopersicon esculentum*), and Old World grape (*Vitis vinifera*). All these affect the following, for which the reverse inoculation experiments were not made: Lettuce (*Lactuca sativa*), green gram (*Phaseolus radiatus*), tobacco (*Nicotiana tabacum*), squash (*Cucurbita moschata*), cucumber (*Cucumis sativus*), and muskmelon (*C. melo*).

Bessey concludes that, "The various families of plants represented in the foregoing list and the fact that the infections were obtained easily and very pronouncedly would seem to indicate the nematode causing root-knot of the plants experimented with, including some of those most generally affected in the field, is not as yet very markedly differentiated into strains peculiar to certain hosts." The results of the present experiment, so far as they go, seem to confirm this view. A large number of sources of *Heterodera radicum* were not available, but the different sources which were available were utilized. The experiment has not progressed far enough to develop new sources. The results of cross-inoculation experiments are summarized in table 1. Infection took place readily in each case reported. The culture media mentioned in the table are described in another paragraph of the article.

TABLE 1—Results of cross-inoculation experiments.

Plant under test	Source of nematodes	Culture medium	Where grown
Cantaloupe varieties	Gardenia plants	Soil	Greenhouse
Whippoorwill cowpea	Sesbania "	Pfeffer's Nutrient Solution.	Laboratory
" "	Tomato plants	Pfeffer's Nutrient Agar	Laboratory
" "	" "	Sand	Laboratory
Tomato varieties	Gardenia plants	Soil	Greenhouse
Striped tomato	" "	Pfeffer's Nutrient Solution	Laboratory
Red Cherry tomato	" "	Pfeffer's Nutrient Solution	"
Red Currant "	" "	Sand	"

Infection took place in each case. The sand cultures were supplied with Pfeffer's Nutrient Solution. See tables 3 and 4 for cantaloupe and tomato varieties.

PLANTS FOUND TO BE INFECTED BY *HETERODERA RADICICOLA*

A number of different plants were sent to the writer for examination during the year. I have included all of those which showed positive infection in table 2. Some of these have been reported by other writers

TABLE 2—*Plants affected by Heterodera radicicola.*

Name	Locality obtained from
<i>Begonia</i> sp.	Greenhouse Berkeley, California
<i>Gardenia</i> sp.	" " "
* <i>Lotus corniculatus</i>	Field in Imperial Valley, Calif.
<i>Medicago hispida reticulata</i>	" " " " "
* <i>Phaseolus max</i> (Mung bean)	" " " " "
" " " "	Field in Fresno Co., California
* <i>Pisum sativum</i>	Field in Imperial Valley, Calif.
<i>Prunus persica</i>	Berkeley, Calif.
<i>Sesbania macrocarpa</i>	Field in Imperial Valley, Calif.
<i>Soja max</i> (Mammoth Yellow Soybean)	" " " " "
" " (Ito San Soybean)	" " " " "
" " (Virginia Soybean)	" " " " "
" " (" ")	Field in Fresno Co., California
* <i>Stizolobium deeringianum</i> (Velvet bean)	" " " " "
* <i>Trifolium alexandrinum</i>	Field in Imperial Valley, Calif.
" " <i>Muscovi</i>	" " " " "
" " <i>Saidi</i>	" " " " "
* <i>Trigonella foenum graecum</i>	" " " " "
<i>Vicia agritinense</i>	" " " " "
<i>V. biennis</i>	" " " " "
<i>V. dasycarpa</i>	" " " " "
* <i>V. faba</i>	" " " " "
* <i>V. fulgens</i>	" " " " "
<i>V. hybrida</i>	" " " " "
<i>V. ludoviciana</i>	" " " " "
<i>V. pannonica</i>	" " " " "
<i>Vicia peregrina</i>	" " " " "
<i>V. polyphylla</i>	" " " " "
* <i>V. sativa duna</i>	" " " " "
" " <i>leucosperma</i>	" " " " "
<i>V. stricta</i>	" " " " "
<i>V. villosa</i>	" " " " "
<i>Vigna unguiculata</i> (Blackeye)	" " " " "
" " (Brabham)	Berkeley and Imperial Valley
" " (Iron)	" " " " "
" " (New Era)	Fresno and Imperial Valley
" " (Whippoorwill)	Berkeley and Imperial Valley

but have been included as confirmatory evidence. In every case, living organisms were found besides the characteristic nematode galls or swellings on the roots. Plants which were not infected have not been listed because we have no way of judging as to immunity unless the plants have been grown under controlled conditions.

VARIETIES OF CANTALOUPE AND TOMATOES FOUND TO BE
SUSCEPTIBLE TO *HETERODERA RADICICOLA*

Sixty-seven distinct varieties of cantaloupes were found to be susceptible to nematode attack. Ninety-eight separate varieties and eight controlled hybrids of tomatoes were also found to be susceptible. In tables 3 and 4, I have listed the names of these varieties and the localities where they were obtained. The varieties were obtained from widely separated localities, as shown in the table. In many instances, samples of a given variety, like Earliana, were obtained from several different localities. Some samples were obtained from one party who had in turn received them from several different sources. In the case of nematode infection, the locality from which the variety was obtained had no effect on resistance, since no biological strains of *Heterodera radicicola* are known. All of the varieties and all of the samples of these varieties of cantaloupes and tomatoes listed in tables 3 and 4 were found to be susceptible to *Heterodera radicicola*.

In order to measure relative degrees of resistance, the plants must be grown under normal conditions, where they can complete their normal life cycle. The plants in the present experiment were largely grown in pots under greenhouse conditions, although a few varieties were grown under field conditions, and a few varieties were grown in the laboratory. In every case, however, the test was of value for estimating immunity or susceptibility rather than relative degrees of resistance. The section on Cross-Inoculation Experiments lists the sources of nematodes for the greenhouse and laboratory experiments. A few tomato varieties were grown on an infected piece of land at the U. S. Plant Introduction Gardens at Chico, Calif., but frost killed the plants before they completed their normal life cycle. Observations were made on the Pollock 10-25 cantaloupe in the Imperial Valley, but no data can be given on resistance, due to lack of controlled conditions. For this reason, the detailed data for each variety has not been presented. The essential fact of the test is that every variety examined is at least susceptible to the attack of *Heterodera radicicola*.

TABLE 3—*Varieties of cantaloupes found to be susceptible to Heterodera radicicola.*

Variety	Culture no.	Locality obtained from
Unnamed	48	Pullman, Washington
"	49	" "
"	51	" "
Acme	9	San Francisco, California
Admiral Togo	60	Detroit, Michigan
Angella	47	Twin Falls, Idaho
Armenian	20	Los Angeles, California
Banana	23	" " "
Bay View	6	San Francisco, California
Blenheim Orange	54	Philadelphia, Pennsylvania
" "	78	Reading, England
Burrell's Gem	8	San Francisco, California
California Beauty	22	Los Angeles, California
Casaba Mixture	19	" " "
" "	25	" " "
Champion Market	65	Detroit, Michigan
Defender	63	" "
Egyptian Deity	35	College Park, Maryland
Emerald Gem	11	San Francisco, California
Empress	80	Reading, England
Extra Early Hackensack	13	San Francisco, California
Extra Tip Top	75	Indianapolis, Indiana
Fordhook	56	Philadelphia, Pennsylvania
Gautier's Pineapple	29	Los Angeles, California
Gold Lined Rocky Ford	14	San Francisco, California
Golden Beauty Casaba	4	San Francisco, California
Golden Eagle	58	Philadelphia, Pennsylvania
Hackensack	10	San Francisco, California
Half Hardy Green Fleshed	81	Reading, England
Half Hardy Scarlet Fleshed	82	" "
Honey Dew	42	Urbana, Illinois
" "	67	Lexington, Kentucky
Honey Dew, Hybrid Casaba	16	San Francisco, California
Hoodoo	44	Urbana, Illinois
"	64	Detroit, Michigan
Improved Hybrid Casaba	27	Los Angeles, California
Indiana Cantaloupe	33	College Park, Maryland
Jenny Lind	21	Los Angeles, California
" "	43	Urbana, Illinois
" "	62	Detroit, Michigan
King George	85	Reading, England
Large Hackensack	26	Los Angeles, California
Maryland Gem	74	College Park, Maryland
Maryland Genus	32	" " "
Mixed Cantaloupe	86	Reading, England
Montreal Green Nutmeg	38	Urbana, Illinois
" " "	69	Lexington, Kentucky

TABLE 3—*Continued*

Variety	Culture no.	Locality obtained from
Montreal Market	15	San Francisco, California
Montreal Market Nutmeg	41	Urbana, Illinois
Netted Rock	30	Los Angeles, California
Osage	24	Macdonald College, Quebec
Osage Gem	17	Los Angeles, California
Paul Rose	18	Los Angeles, California
" "	61	Detroit, Michigan
Persian	2	San Francisco, California
Pollock 10-25	7	" " "
Pollock 10-25 Selections	1	Imperial Valley, California
Rocky Ford	3	San Francisco, California
" "	46	College Park, Maryland
" "	68	Lexington, Kentucky
Rosa	71	Waverly Mills, South Carolina
Royal Jubilee	91	Reading, England
Salmon Tint Pollock-25	57	Philadelphia, Pennsylvania
Santa Claus	28	Los Angeles, California
" "	37	College Park, Maryland
Scarlet	92	Reading, England
Selection of Buskuks Gem	34	College Park, Maryland
Spicy	59	Philadelphia, Pennsylvania
Superlative	93	Reading, England
Texas Camionball	70	Lexington, Kentucky
Ten Twenty Five	39	Urbana, Illinois
Tip Top	12	San Francisco, California
" "	40	Urbana, Illinois
Tait's Thoroughbred Bottomly Muskmelon	52	Norfolk, Virginia
Thoroughbred Knight Muskmelon	53	" "
Universal	95	Reading, England
Vaughan's Original Osage	45	Urbana, Illinois
Windsor Castle	55	Philadelphia, Pennsylvania
Winter Pineapple	5	San Francisco, California

TABLE 4—*Varieties of tomatoes found to be susceptible to Heterodera radicicola*

Variety	Culture no.	Locality obtained from
Acme	266	Moscow, Idaho
Adirondack Earliana	267	" "
Albino	305	Lincoln, Nebraska
"	306	" "
Anne	145	Berkeley, California
Atlantic Prize	23	Los Angeles, California
" "	268	Moscow, Idaho

TABLE 4—*Continued*

Variety	Culture no.	Locality obtained from
Baur's Yellow	30	College Park, Maryland
" "	34	" " "
B.C. 2-A	50	" " "
" "	51	" " "
Beauty	15	San Francisco, California
Best of All	303	Reading, England
Bonnie Best	12	San Francisco, California
" "	26	Durham, New Hampshire
California Wild	125	Berkeley, California
Canner	48	College Park, Maryland
Chalk's Early Jewel	14	San Francisco, California
Cherry	46	College Park, Maryland
Coreless	53	" " "
"	169	Farmington, Utah
Cuke X Coreless Borghese	45	College Park, Maryland
Dwarf Aristocrat X Buckeye State	39	" " "
" " " "	49	" " "
Dwarf Champion	13	San Francisco, California
" "	260	Waverly Mills, South Carolina
" "	270	Moscow, Idaho
Dwarf Giant	271	Moscow, Idaho
Dwarf Ponderosa	272	" "
Dwarf Quarter Century	176	Farmington, Utah
Dwarf Stone	131	Philadelphia, Pennsylvania
" "	177	Farmington, Utah
Ealibell A	273	Moscow, Idaho
Ealibell B	274	" "
Earlana	16	San Francisco, California
"	73	State College, Pennsylvania
"	146	Berkeley, California
"	275	Moscow, Idaho
"	276	Philadelphia, Pennsylvania
"	277	Moscow, Idaho
"	278	New York, N. Y.
"	279	Moscow, Idaho
"	280	" "
"	281	" "
"	282	" "
"	283	New York, N. Y.
Earliest Market	284	Moscow, Idaho
Early Detroit	11	San Francisco, California
Early June	286	Moscow, Idaho
Early Market	285	" "
Early Prolific	287	" "
Early Ruby	288	" "
Fiascove	83	Mexico by U. S. D. A.

TABLE 4—*Continued*

Variety	Culture no.	Locality obtained from
Ficarazzi	264	Columbia, Missouri
Fifty Day	289	Moscow, Idaho
First Early	22	Los Angeles, California
Fusarium Wilt Resistant	29	Georgia
Globe	28	"
Golden Dwarf Champion	290	Moscow, Idaho
Golden Queen	9	San Francisco, California
" "	77	Santa Ana, California
Hardy Jewel	147	Berkeley, California
Hummer	32	College Park, Maryland
Individual Coreless Borghese	54	" " "
John Baer	5	San Francisco, California
" "	291	Moscow Idaho
June Pink	292	" "
Kansas Standard	293	" "
Large Meaty	40	College Park, Maryland
Large Pear	44	" " "
Large Plum	52	" " "
Large Yellow	238	Reading, England
Livingston's Coreless	20	Los Angeles, California
Livingston's Favorite	24	" " "
Livingston's Globe	8	San Francisco, California
" "	72	Columbia, Missouri
Louisiana Pink	261	Davis, California
Maryland Slicing	31	College Park, Maryland
" "	37	" " "
Maryland Solid	27	" " "
Matchless	6	San Francisco, California
Matchum	84	State College, Pennsylvania
Missouri 307	254	Columbia, Missouri
" 309	255	" "
" 313	256	" "
" 314	257	" "
" 317	258	" "
Moore's Early	294	Moscow, Idaho
Murray's New Perfection	36	College Park, Maryland
Narduke	57	Lexington, Kentucky
Norton	56	" "
"	88	Santa Ana, California
"	140	" " "
"	205	Farmington, Utah
Orobia	76	Mexico through U. S. D. A.
"	265	Columbia, Missouri
Payne's Victory	25	Los Angeles, California
Peach	21	" " "

TABLE 4—*Continued*

Variety	Culture no.	Locality obtained from
Pierrette	307	Paris, France
P-L-M	308	" "
Ponderosa	7	San Francisco, California
"	108	Manhattan, Kansas
"	143	Philadelphia, Pennsylvania
Porto Rico Wild × (Cherry × Maryland Solid)	47	College Park, Maryland
Porto Rico Wild × (Cherry × Red Rock)	35	" " "
Precocis	82	Mexico through U. S. D. A.
Princess of Wales	304	Reading, England
Red Cherry	81	Santa Ana, California
Red Currant	63	Santa Ana, California
Red Forty Day Genoa	263	Columbia, Missouri
Red Head	295	Moscow, Idaho
Red Pear	1	San Francisco, California
" "	65	Santa Ana, California
" "	139	San Francisco, California
Red Riding Hood	297	Moscow, Idaho
" " "	298	" "
" " "	299	" "
Red Rock	19	Los Angeles, California
" "	296	Moscow, Idaho
Reich × Coreless	33	College Park, Maryland
Rosso	75	Mexico through U. S. D. A.
San José Canner	4	San Francisco, California
Selection	41	College Park, Maryland
Self Pruning	300	Moscow, Idaho
Stone	17	San Francisco, California
Striped Tomato	78	Berkeley, California
Sunrise	269	Moscow, Idaho
Tister's Empire	55	College Park, Maryland
Trophy	18	San Francisco, California
Victoria Whole Solid	301	Moscow, Idaho
Yellow Cherry	86	Santa Ana, California
Yellow Cherry	302	Moscow, Idaho
Yellow Pear	2	San Francisco, California
" "	64	Santa Ana, California
" "	138	San Francisco, California
Yellow Plum	3	" " "
" "	137	" " "
" "	142	Santa Ana, California
Yellow Ponderosa	66	" " "
Yep	69	Macdonald College, Quebec
"	148	" " "

TABLE 5—Controlled hybrid tomatoes found to be susceptible to *Heterodera radicum*.

Red Currant	22-63 P-21 × Red Cherry	22-81 P-20	22.3 F ₁ -6
Yellow Cherry	22-86 P-8 × " "	22-81 P-8	22.3 F ₁ -2
" "	22-86 P-8 × Red Currant	22-63 P-21	22.3 F ₁ -1
Yellow Pear	22-64 P-30 × Earliana	22-70 P-8	22.3 F ₁ -13
" "	22-8-2 P-1 × Red Cherry	22-81 P-20	22.3 F ₁ -11
" "	22-8-2 P-2 × Red Currant	22-63 P-21	22.3 F ₁ -9
" "	22-8-2 P-1 × Yellow Plum	22-8-3 P-1	22.3 F ₁ -10
Yellow Plum	22-62 P-31 × Red Currant	22-63 P-21	22.3 F ₁ -12

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A SPECIES OF OLPIDIUM PARASITIC IN THE ROOTS OF TOMATO, TOBACCO AND CABBAGE

MATHILDE BENS AUDE

WITH FIVE FIGURES IN THE TEXT

Woronin¹ in 1878 found a parasitic fungus in the roots and hypocotyl of blighted cabbage seedlings and named it *Chytridium brassicae*. The organism was later transferred to the genus *Olpidium* by Dangeard,²

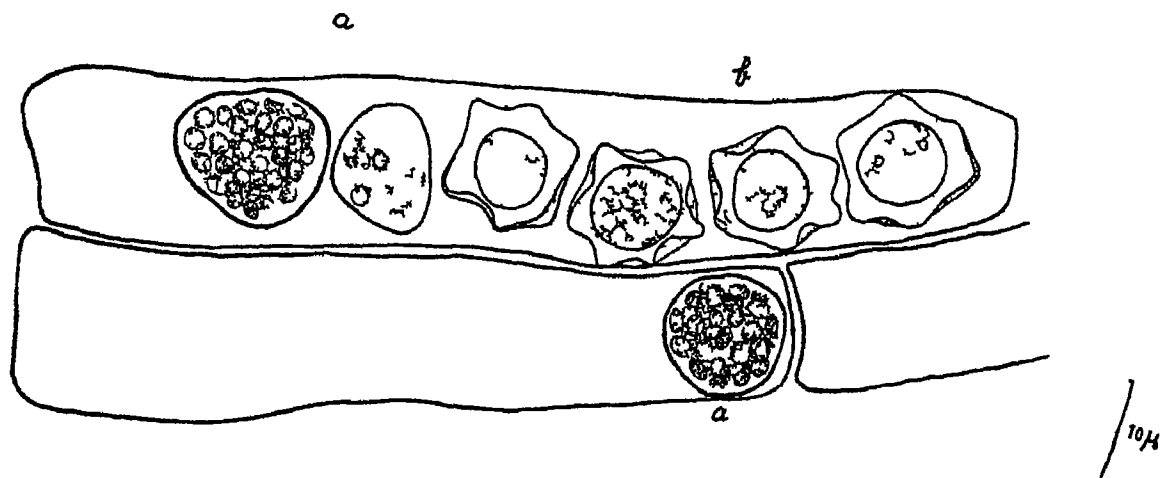


FIG 1 Cross section through cortical cells of tomato root containing zoosporangia (a) and resting spores (b) of *Olpidium* sp (Camera lucida drawing, host cells semi-diagrammatic)

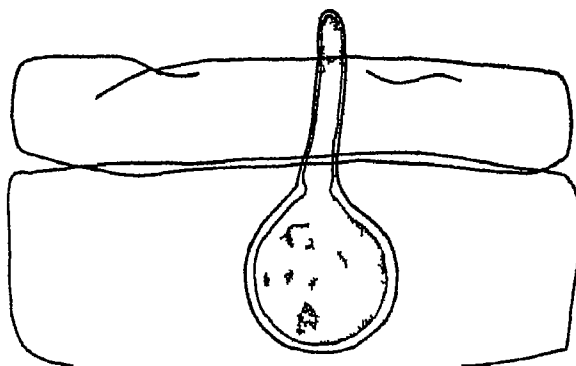


FIG 2 Cross section of zoosporangium with neck-like structure having reached the surface of the parasitized cabbage root. The protoplasm is dividing into zoospores (Camera lucida drawing, the host cells semi diagrammatic)

and is now known as *Olpidium brassicae* (Wor) Dangeard. The pathogenicity of the fungus has been assumed without any experimental

¹ Woronin, Jahrb Wiss Bot 11 556 1878

² Dangeard, Ann Scienc Nat Ser 7, 4 327 1886

evidence. Peters and Schwarz,¹ in a bulletin on tobacco diseases, report that a yellowing and blighting of tobacco seedlings caused by *Olpidium brassicae* has often been observed in Dalmatia and Gallizia. Root cells of tobacco invaded by *Olpidium* are figured after Preissecker. The writer has not been able to find in the bulletin or anywhere else the reference to Preissecker's work, and has not seen the original paper.

Since the writer has found no case of *Olpidium*, as a root parasite, reported in American literature, it seems worth while to give a brief description of an *Olpidium* infection of the roots of several plants observed in the green house while working with another root disease of tomato and tobacco at the University of Wisconsin.

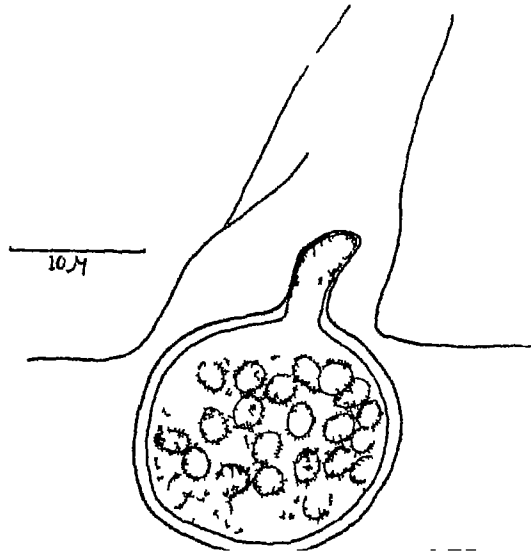


FIG. 3. Cross-section through a zoosporangium parasitic in a cabbage root cell. The neck is growing into a root hair. (Camera lucida drawing, the host cell semi-diagrammatic)

The infection was first found on tomatoes. Tobacco and cabbage plants grown in the same soil became readily parasitized. Infected plants developed quite normally. Even the roots looked healthy save for some areas of superficial and very slight discoloration. Seeds of cabbage and tobacco sown in infected soil produced quite normal seedlings, yet upon examination the roots proved to be heavily infected. In its morphology the fungus found in roots of tomato, tobacco, and cabbage corresponds very closely to the description of *Olpidium brassicae* given by Woronin. The zoosporangia are found singly or several together in cortical cells. They are most abundant in the outer layer,

¹ Peters and Schwarz, Mitt K. Biol Anst. f. Land- u Forstwirtsch. Heft 13:10. 1912.

but are also often found at a considerable distance from the surface, as far inward as the fourth or fifth row of cells. These bodies are spherical or else slightly elongated in the direction of the longer axis of the host cell. The walls are smooth, relatively thin, and very permeable to stains. The protoplasm, dense in young sporangia, divides when maturity approaches into areas corresponding to the future zoospores. At maturity the zoosporangia produce a neck-like structure through which the zoospores are liberated. In our preparations a plug of dense protoplasm was always present at the apex of these structures before

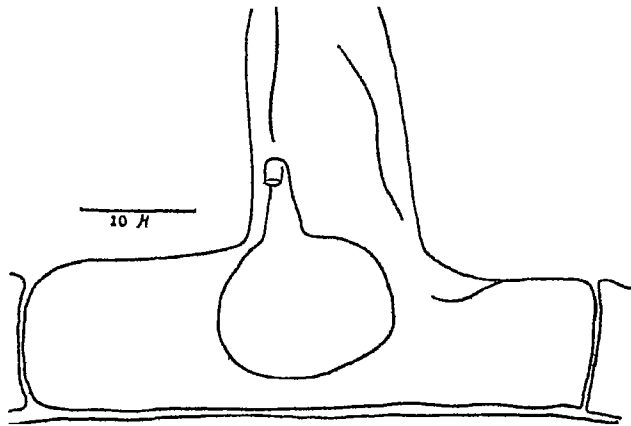


FIG. 4. Zoosporangium having discharged the zoospores, apparently into a root hair. (Outline of zoosporangium drawn with camera lucida, host cells semi-diagrammatic)

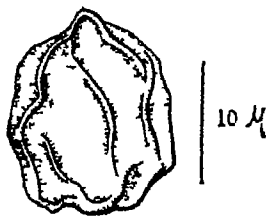


FIG. 5. Surface view of resting spore teased out of host cell

rupture. The latter vary greatly in length from one zoosporangium to another. Most of them grow through one or several cells to the surface of the host (Fig. 2) and apparently discharge their contents directly into the soil. Others never reach the surface of the host and probably liberate the zoospores into the cortical cells or root hairs in which they terminate (Figs. 3 and 4). According to Woronin the zoospores of *Olpidium brassicae* are spherical, with one single anterior flagellum. Having never seen mature zoospores we do not know if this is also the case for the *Olpidium* in question. Resting spores similar to those

described by Woronin were found abundantly in the outer cell layer of roots, often at the base of root hairs. The resting spores are white or light yellow with a thick wall impenetrable to thionin; the protoplasm is colorless, granular and rich in oil droplets. Young resting spores are slightly polygonal in contour, older ones become wrinkled and covered with ridges (Fig. 4), appearing star-shaped in cross section (Fig. 1). Characteristic thick-walled resting spores were observed not exceeding 6 to 7 microns in diameter. The larger ones found attained a diameter of 30 microns. The ultimate development of the resting spores has not been observed.

Judging by the figures and short description of Woronin, the *Olpidium* described in this note seems to be identical with *Olpidium brassicae* (Wor.) Dangeard. The serious pathogenic action attributed to this fungus by Woronin and other above mentioned authors was not observed. Further investigation would be necessary in order to ascertain whether the earlier writers were justified in attributing to the *Olpidium* present in the lesions the blighted condition of cabbage and tobacco seedlings, and whether the *Olpidium* observed in Wisconsin is really identical in every respect to the European *Olpidium brassicae* (Wor.) Dangeard.

UNIVERSITY OF WISCONSIN.

A NEW HOST FOR BACTERIUM MALVACEARUM

J G BROWN AND FREDERICK GIBSON

WITH TWO FIGURES IN THE TEXT

In the autumn of 1922 it occurred to the writers that cross-inoculations of Pima-Egyptian cotton and Arizona wild cotton (*Thurberia thespesioides*), with the angular leaf spot organism *Bacterium malvacearum*,



FIG 1 Young plant of Arizona wild cotton *Thurberia thespesioides*

should prove interesting Accordingly seeds of *Thurberia* were planted in a bed in the greenhouse, which already contained plants of the Pima-Egyptian strain of *Gossypium*, and inoculations were later made which are briefly described below

Thurberia thespesioides is a perennial shrub varying in height from four or five feet to ten feet or more. It grows in washes, arroyos, canyons, and on caynon walls in southeastern Arizona, at altitudes of two thousand to seven thousand feet. The leaves, which are mostly tri-lobed, are thinner and more delicate and the stems woodier than those of cultivated varieties of *Gossypium*.

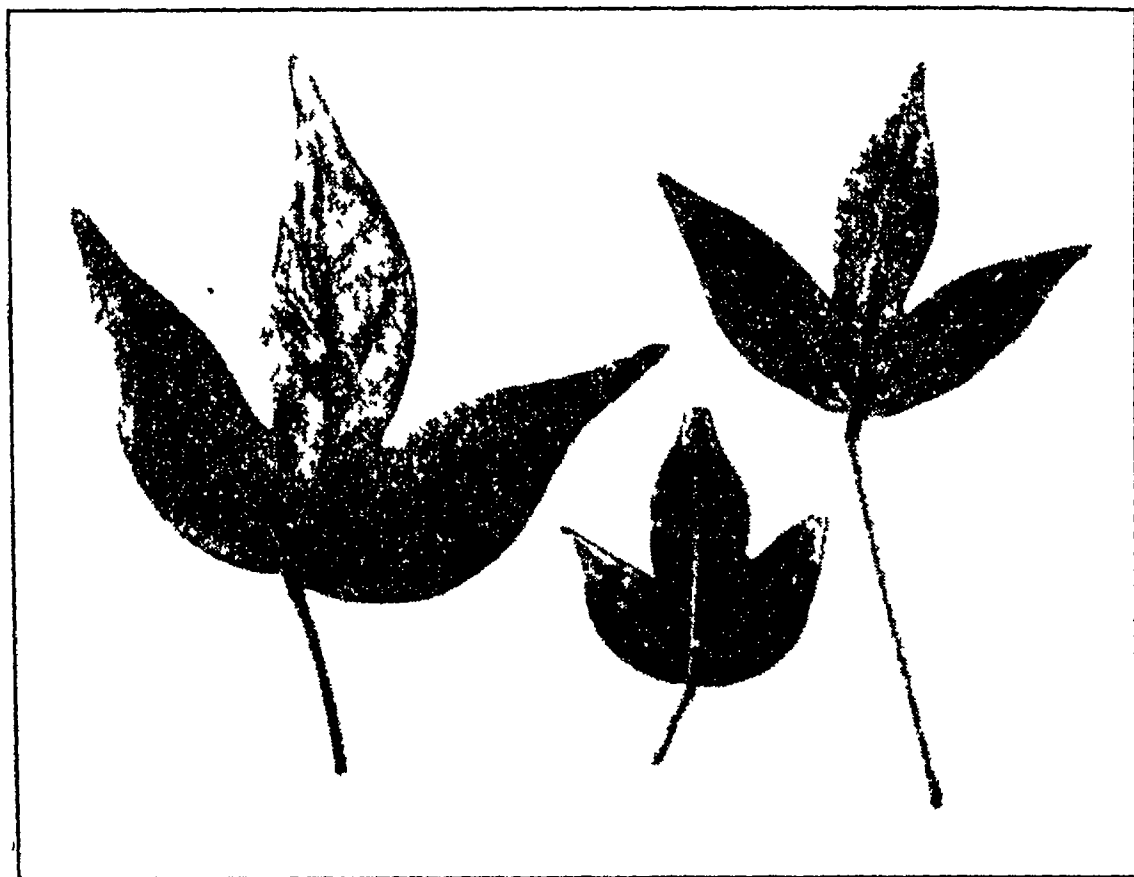


FIG 2 Leaves of Arizona wild cotton showing spots infected with the angular leafspot organism *Bacterium malvacearum*. The leaf on the left has a dying middle lobe; that on the right has a marginal infection.

When the plants of *Thurberia* in the greenhouse were about one foot high they were inoculated with a pure culture of *Bacterium malvacearum* obtained from Pima-Egyptian cotton grown on the Salt River Valley Farm of the Arizona Agricultural Experiment Station. Inoculations were made by spraying and also by rubbing the inoculum on the leaf surfaces with the fingers. At the same time the Pima-Egyptian plants were treated similarly. Infections resulted in both kinds of plants, although they were slower to appear in the *Thurberia* plants. From the infected areas, the angular leaf spot organism was isolated.

The infected spots on the wild cotton were generally confined to the leaves and were usually found along the veins as shown in figure 1, whether the leaves were young or mature. In a few leaves the infected areas were marginal, and in some others they were intervenar and exactly similar to the angular spots on cultivated cotton caused by *Bacterium malvacearum* except that they were smaller because of the smaller size of the intervenar spaces in leaves of *Thurberia*. Eventually the infected area became dark brown, the tissues yellowed beyond the diseased region, and the leaf dropped. Shedding of the leaves of *Thurberia* appears to result from much less infection than is the case with cultivated varieties of true cotton. Only one typical black arm lesion was found, which was located on a green twig.

ARIZONA AGRICULTURAL EXPERIMENT STATION,
TUCSON, ARIZ.

DISCOVERY OF CURLY LEAF OF SUGAR BEETS IN THE ARGENTINE REPUBLIC

P. A. BONCQUET

During the season from August 1916 till February 1917, the writer was investigating the possibilities of the sugar beet industry in the Argentine Republic. This study was started under the direction of the Franklin & Herrera Land Company of Buenos Aires. Three stations were established: one in San Isidro, one in Guatrache and one in Colonia Alvear. Small plots of beets were grown and the development of the beet noticed.

The curly leaf of beets was first observed at San Isidro, a suburb of Buenos Aires. The plants affected were a variety of small beet growing spontaneously along the Rio de la Plata. The black rings in the root, the protuberances on the leaves, and the dwarfing of the heart leaves were characteristic signs of the presence of the disease. The discovery, however, was so unusual and so unexpected, that it did not convince the writer to his complete satisfaction. A search for more evidence was at once undertaken and a great number of vegetable gardens around Buenos Aires, where garden beets were grown, were visited. In most places, where garden beets were found, the same symptoms of the disease were observed. But, as it was winter then, the disease was rather of a mild type. Efforts were made to find specimens of *Eutettix tenella* Baker, the leaf hopper whose sting incites curly leaf. A few were captured and examined. They had the same appearance as those which the writer captured in Berkeley, California, in 1914. They were dark grey, full-grown and ready for oviposition. Dark specimens of the same kind have repeatedly been observed during the winter of 1915 in the Salinas Valley, California. There, they were hiding under the grass in the irrigation ditches and on sunny days became active on those sides of the ditches which were exposed to the sun. This winter form of the insect was observed not only in the beet gardens, but also along the Rio de la Plata at San Isidro, the seasonal and regional conditions being very similar to those in Berkeley and in the Salinas Valley in California.

Upon his arrival in Colonia Alvear (Western Argentina), the writer visited the experimental beet plots of the Colonia Alvear Land Company. The beets which had been left from the previous year had all gone to seed. However, the signs of curly leaf were evident. The protuberances on the leaves and the rings in the roots, characteristic of the

disease, were noted. Microscopic examination revealed also the characteristic ring-shaped and S-shaped bodies in the necrotic phloem bundles.

By this time, hardly any doubt could remain as to the presence of the disease in the southern republic. A few specimens of *Eutettix tenella* were also discovered there at this time. Nevertheless, a careful study was undertaken in order to corroborate our conclusions. Small beet plots were sown in Colonia Alvear, where the writer resided during the following summer (November–February). These plots were kept under close observation. Efforts were made to locate the breeding grounds of the insect in these regions. It was found at this time that the insect was present quite abundantly on the mud flats of the river Atuel, which passes through Colonia Alvear. Here the vegetation was of the salt marsh type and vegetation was more abundant than in the desert. However, a few specimens of *Eutettix tenella* were also found in the fields. The beets in the experimental plots came up and grew luxuriantly, as the soil is very fertile. No disease was observed until the beets were fully covering the ground. At this time a few beets began to have the watery translucent veins on the youngest leaves. Five days later, more than fifty per cent of the beets had developed the disease with all the characteristics which could be desired for identification. Ten days later only a few beets could be found which did not show some of the symptoms. So bad was the infection that a great number of the beets died.

By this time, *Eutettix tenella* was abundant both on the beet plots and in the adjacent bean fields. They were all full-grown specimens and rather light grey in color. On the desert vegetation, which was now drying up, many nymphs were to be found. The condition was about the same as the writer has observed many times around Visalia and also in the Salinas Valley (California).

The habitat and the habits of *Eutettix tenella* appear to be the same in South America as in North America. From Buenos Aires southward along the coast to Bahia Blanca, westward across the continent to Colonia Alvear and Mendoza, one meets climatic and regional conditions about the same as along the coast and in the valleys of California. *Eutettix tenella* is met with over those vast regions and this seems to be in its natural habitat. The habits of the insect and its relation to plant life seem to be the same in both continents. The insect moves from one aspect of vegetation to the other as they succeed during the season. It wants fresh growth for its nourishment and the plants which have the youngest green are usually the plants upon which it feeds. If in the middle of a desert a cultivated beet field appears at the time the

natural vegetation is drying up, the *Eutettix tenella* moves towards this young growth as occurred on our experiment plot in Guatrache.

Specimens of *Eutettix tenella* were caught and preserved in alcohol. They were identified by Mr. E. P. Van Duzee of the California Academy of Sciences, San Francisco, as being identical with our species, and specimens were deposited in the collection of the Academy. The insect in its local habitat seems to migrate from the foothills of the Andes and to invade the desert region following up the green vegetation after the winter rains have ceased. The insect has been found around Buenos Aires, in Guatrache, Alpachiri and Bahia Blanca (South-East), in Colonia Alvear and Mendoza (West), covering the major part of the temperate and sub-tropical zones of the Argentine Republic.

Specimens of diseased beets were also brought to the University of California for determination of the disease by Professor R. E. Smith of the Division of Plant Pathology. The insects and diseased plants were also examined and identified by Dr. H. H. Severin of the Division of Entomology.

PHYTOPATHOLOGICAL NOTES

Apple bitter-rot cankers in the eastern United States. In U. S. Department of Agriculture Bulletin 684, published in 1918, the writer stated that after careful search he had been unable to find bitter-rot cankers in the apple orchards of the eastern United States. He also noted that he had found no record of the occurrence of bitter-rot cankers in this region. On April 4, 1921, Dr. Errett Wallace of the Bureau of Plant Industry, U. S. Department of Agriculture, collected near Cornelia in northern Georgia, two diseased apple branches which he turned over to the writer. These branches bore typical bitter-rot cankers which, after remaining in moist chambers for 18 hours, were covered with the pinkish acervuli so characteristic of *Glomerella cingulata*. The spores, also, were in every way typical. On artificial media growth and development were the same as for authentic cultures from diseased fruits. Inoculation of healthy apples produced typical bitter-rot. In so far as the writer has knowledge of the subject, this is the first time that bitter-rot cankers have been reported from an Atlantic Coast State. The cankered branches have been deposited in the Office of Pathological Collections, Bureau of Plant Industry, U. S. Department of Agriculture. —JOHN W. ROBERTS.

Plum blotch. The attention of those interested in the plum disease caused by *Phyllosticta congesta* and described by the writer in the Journal of Agricultural Research, vol. 22, is called to the following paragraph on page 95 of the Cornell Agricultural Experiment Station Bulletin 38, "The cultivated native plums and cherries" by L. H. Bailey, published in 1892.

"A fruit spot (Fig. 12) has been sent me by T. V. Munson, Denison, Texas, on the Golden Beauty. The disease is said to be serious in some years. It does not injure the fruit greatly except to disfigure it and to render it unmarketable. I submitted the specimens to Prof. J. E. Humphrey, of the Mass. Agricultural College, who found the disease to be a phoma, but the exact species could not be determined. It is, no doubt, somewhat allied to the black-rot of the grape, and the treatment used for grapes should be tried upon the plum."

The disease mentioned by Bailey is undoubtedly blotch. Fig. 12 is a line cut reproduction of a pen drawing showing a plum with numerous spots closely resembling blotch. As *Phyllosticta* and *Phoma* are closely

allied genera and as forms are commonly placed in one or the other according to their situation with regard to the host, Humphrey's identification of the fungus is easily understood. The very close resemblance between the *Phoma* stage of the grape black-rot pathogen and *Phyllosticta congesta* probably suggested the possibility of relationship between the two diseases. The Golden Beauty is a variety propagated from a plum found growing wild in western Texas and belongs to the Wayland group of *Prunus hortulana*. The finding of the disease on this variety as long ago as 1892 suggests the possibility that it may be indigenous and may have passed from wild plums to cultivated varieties and to other species such as *Prunus triflora* on which it was found in Georgia. This possibility is further supported by the fact that *Phyllosticta congesta* was originally described by Heald and Wolf as occurring on plum leaves in Texas.—JOHN W. ROBERTS.

Brown rot of apple. During the past winter the writer was making cultures from a considerable number of Stayman winesap apples for the purpose of securing cultures of *Glomerella rufomaculans* and was very much surprised to find that in nearly every case the decay was due either to *Botrytis* or *Sclerotinia*. Eight strains of the former and three of the latter were grown on four different media and carefully studied. In the case of *Botrytis* the strains showed very slight variations; in fact there was not as much variation between the different strains when grown on the same medium as between cultures of the same strains on different media. The conidiospores were mostly spherical and about $16\ \mu$ in diameter but some of them were elongated and measured $16 \times 20\ \mu$. Sclerotia were formed most abundantly on the oatmeal agar and ranged from one mm. in diameter to those that were 5×8 mm. in size. The Sclerotia cultures showed a great variation in vigor of growth and in size of conidiospores. The weakest grower developed spores ranging from 8×12 to $6 \times 16\ \mu$; the next stronger developed spores measuring from 20×20 to $24 \times 36\ \mu$; while the strongest grower developed spores measuring from 16×20 to $24 \times 28\ \mu$.—MEL. T. COOK.

A greenhouse disease of melons. During the winter of 1922-23, the attention of the writer was called to a disease of muskmelons in a greenhouse in northern New Jersey. The seeds were imported from England and the plants were grown on trellises, each plant being allowed to bear only one fruit. The disease was designated as "streak" by the gardener because that term very aptly described the symptoms. The gardener informed the writer that the first evidence of the disease would appear at any point on the vine and work in both directions as a dead brown

streak in the stem and frequently work out into the leaves. Pure cultures were obtained of a *Fusarium* very similar to *F. vasinfectum* but no inoculations were made to determine its pathogenicity. The question as to whether the organism was in the soil or in the seed could not be answered but it was probably the former.—MEL. T. COOK.

A serious disease of wheat caused by Sclerotium rhizodes in Idaho.—Early in the spring of 1922 specimens of a sclerotium disease on winter wheat was sent to the writer from Fremont County, Idaho. The disease was reported to be generally present in wheat fields aggregating about 300 acres and appeared to be causing considerable damage to wheat over that area. The original report of the disease stated that effected plants were covered with yellow or brown bodies and that when the snow melted these plants were covered with a mouldy growth. The disease appeared to be most severe where the snow had remained on the ground late in the spring. As the spring was exceedingly backward many farmers had attributed the injury to the heavy blanket of snow remaining so long on the ground.

Many of the plants which were sent in to the Experiment Station at Moscow were entirely dead. Others were only partially dead, the inner leaves still remaining a light green. The lower portions of these green leaves were often blackened and in some cases brown spots appeared upon these leaves. The bodies referred to above which according to reports were yellow or brown earlier in the season were found to be sclerotia and were a very dark brown or in most cases black when the specimens were received. They were scattered over the surfaces of the dead and dying leaves to which they were attached very securely. It was found practically impossible to separate them from the leaf surface without tearing the leaf. They were also present on the roots for one or two inches below the crown, and were found in great numbers embedded inside of the leaf sheath. They were about the size of the head of a small pin, spherical, or somewhat oval and flattened when dry and wrinkled or corrugated over their surfaces. They were very numerous on dead plants and on the dead leaves of plants which were partially killed. Twenty-five sclerotia were counted in one inch of the leaf of a dead plant.

As the writer was not familiar with the disease and did not have available facilities for determining it, specimens were sent to the Office of Pathological Collections of the United States Department of Agriculture for determination. The sclerotia were identified as sclerotia of *Sclerotium rhizodes* Awd. Miss Vera K. Charles of the Office of Patho-

logical Collections states in a letter regarding the matter that a similar specimen was sent in by Prof. R. A. Cooley of the Experiment Station at Bozeman, Montana in 1907

The writer visited the region where the disease occurred late in June and made a rather careful survey of infected fields. The disease was found in a number of fields, the losses in the various fields ranging from a trace to fifty per cent in some cases. The disease appeared in spots in the fields and in these spots the majority of the plants were killed. The old dead plants could still be found on the ground where they had been killed in the rosette stage of growth. Around the margin of these spots as well as scattered thru the fields could be found plants which were only partially killed. These plants usually had but one or two weak stalks which often had failed to produce heads.

Thus far the present season the disease has not been reported. A careful survey will be made for it this year and careful investigation will be undertaken should it appear again.—CHAS. W. HUNGERFORD.

Personals: Dr. John Asbury Elliott, Professor of Plant Pathology in the University of Arkansas and Plant Pathologist for the Arkansas Agricultural Experiment Station, died Thursday, January 18, at the Homeopathic Hospital, Washington, D. C. While at the Boston meetings of the American Phytopathological Society he became ill, and, after coming to Washington, pneumonia developed. The funeral services and burial were held January 20, at Rock Creek Cemetery in Washington, D. C.

Dr. Elliott held degrees from Fairmount College, the University of Kansas and the University of Illinois. From May 1916 to July 1917 he was Assistant Plant Pathologist at the Delaware Experiment Station, and from July 1917, until the time of his death he was the head of the Department of Plant Pathology at the University of Arkansas. He was thirty-five years old and leaves a wife and two children.

The September number of Phytopathology was issued September 18, 1923.

PHYTOPATHOLOGY

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TIME-TEMPERATURE CURVES FOR KILLING POTATO TUBERS BY HEAT TREATMENTS

F. M. BLODGETT

WITH THREE FIGURES IN THE TEXT

The mosaic and leaf-roll diseases of potatoes were early found (8) to be transmitted from year to year by means of the tubers. Since none of the disinfecting solutions used experimentally or in practice has shown the slightest indication of reducing the amount of either of these diseases, and since they may be transmitted by using the juice of diseased plants (4), it may be accepted without much question that the disease producing agent is internal in the tuber. Some method of heat treatment, therefore, would be indicated as offering possibilities of success. The experiments reported here were conducted with particular reference to the two diseases mentioned. Experiments were made both by treating potatoes in a hot water bath and in a hot air oven. Advantage was taken of the opportunity thus offered to formulate an approximation of the conditions of time and temperature necessary to kill potatoes by such treatments. This is believed to be worth recording both as a guide that may be used in the treatment of potatoes by these methods for other diseases and as indicating the conditions under which potatoes may be injured in storage or transit. It is hoped also that the results presented here may give some clue to the effect of varying the temperature in other seed treatments.

The plan was to make experiments over as wide a range of temperatures as practicable. At each temperature selected, treatments were made over a range of time intervals from comparatively short treatments up to treatments long enough to kill the potato. This was deemed necessary and desirable since it is easily conceivable that the time-temperature death curve for the potato might cross the time-temperature death curve for the infective agent of mosaic. Thus the potato might be killed more quickly at one temperature and the infective agent of mosaic more quickly at another temperature. That this is possible is indicated by the results of Miss Chick (3) in the killing of bacteria by hot water. She found that the velocity of disinfection is increased more than 100-

fold per 10° C. rise in temperature in case of *B. typhosus*, 29-fold per 10° C. for *Staphylococcus pyogenes*, and 12-fold per 10° C. for *B. Coli*.

After the treatments, the potatoes were labelled and stored at laboratory temperatures where they either sprouted in a short time or if killed by the treatment dried up or rotted. They were inspected from time to time. If they sprouted a record was taken of the approximate kind and amount of sprouting and the potatoes were planted in the greenhouse to see whether they produced leaf-roll or mosaic plants, according to whether the seed treated was taken from mosaic or leaf-roll plants. If the potatoes were killed by the treatment no effort was made to determine whether the virus was still infective or not. This would have been desirable but the methods of inoculation so far tried did not seem certain enough to make the attempts worth while.

In this manner, the time necessary to completely kill potatoes was sought at each temperature at which tests were made. The time finally accepted as the one sought was one midway between the treatment where the last potatoes sprouted and the first in which no sprouting occurred. This is clearly only an approximation both because the end point is not sharply defined and because the end point found is more or less dependent on how near together the intervals selected for treatment are in this neighborhood.

HOT WATER TREATMENTS

The hot water treatments were made in a water bath in tap water, heated by an electric heater under thermostatic control. Mechanical agitation was supplied in all the later tests.

In making the treatments it was found better to place the potatoes in the bath a few at a time at such intervals that they could be removed all at once to give the series of treatments at the desired intervals. In this way the temperature of the bath was not measurably disturbed by the addition of potatoes.

A sample of the data recorded in one test is given in table 1. A series of twenty-eight such tests were made at different temperatures varying from 34.5° C. to 80° C. A summary of these tests is shown in table 2. As shown in table 1, the end point at 55.5° C. lies between 22 and 26 minutes. In making up summary table 2 the midpoint between these two times, i. e., 24 minutes was chosen as representing the time for killing as nearly as possible from the data at hand.

It is to be noted that the potatoes used in different tests differed in age and variety but the results were so uniform that they are all considered together.

TABLE 1—*Hot water treatment at a temperature of 55.5° C. of blue sprout potatoes affected with leaf-roll.*

Length of treatment	Conditions of potatoes				Progeny
	Mar. 21	Apr. 7	May 15	July 26	
0 min.	sprouted	sprouted			Leaf-roll
2 "	"	"			" "
4 "	"	"			" "
8 "	"	"			" "
14 "	not sprouted	not sprouted	slender sprouts		" "
16 "	"	"	one sprout		" "
18 "	"	"	firm no. sprouts		
22 "	"	"	"	4 weak sprouts	" "
26 "	withered	dead			
32 "	dried up	"			
40 "	black shrunk				

The results are also shown in figure 1 in which temperatures in degrees Centigrade are plotted as abscissas and the logarithms of the times in minutes are plotted as ordinates. When plotted in this way, the points seem to approximate a straight line so that the relation of time to temperature in the killing of potatoes may be expressed by one of the standard forms of equation of the straight line as

$$y + mx + b = 0$$

in which y equals the logarithm¹ of the time and x is the temperature in degrees Centigrade. When this was fitted by the method of least squares the following values were found for the constants m and b .

$$m = .09552 \pm .0026$$

$$b = -6.5896 \pm .0191$$

The equation

$$y + .09552x = 6.5896$$

¹ Wherever logarithms appear in this article the common or Brigs system is used.

TABLE 2—*Time required to kill potatoes at different temperatures in a water bath.*

Temperature in degrees Centigrade	Time in minutes	Disease	Variety	Date
34.5	2310	Mosaic	Bliss	5/9/21
35	2370	"	"	5/16/21
35	2700	"	"	5/3/21
40	480	"	"	5/6/21
40.5	495	Leaf-roll	Rurals	2/10/22
41	570	Mosaic	Bliss	7/14/21
44	370	Leaf-roll	Rurals	2/16/22
50	60	Mosaic	Bliss	3/24/21
50	60	"	"	3/3/21
50	45	"	"	3/11/21
49.7	47.5	Leaf-roll	Rurals	2/7/22
55.5	24	" "	"	2/23/22
56	10	Mosaic	Bliss	5/3/21
59.5	10	"	"	7/3/21
60	4.25	"	"	3/24/21
60	11	"	"	3/4/21
60	12	Leaf-roll	Rurals	2/22/22
65	1.12	Mosaic	Bliss	4/12/21
65	1.25	"	"	3/25/21
25.8	2.00	Leaf-roll	Rurals	2/23/22
70	.25	Mosaic	Bliss	7/25/21
75	.66	"	"	7/22/21
80	.266	"	"	7/25/21

is therefore offered as an empirical approximation of the relation of the time and temperature for killing such potatoes in hot water treatments. The potatoes used in these experiments came from definitely diseased plants either of mosaic or leaf-roll as indicated. The behavior of perfectly healthy tubers, therefore, can not be stated certainly from these tests but judging by the work reported by McCurry (5) and by the results reported later in this paper under hot air treatments it is altogether probable that the curve or equation as given above represents the conditions under which healthy potatoes are killed very nearly if not quite as well as for diseased tubers.

The deviations of some of the killing points found from this line are rather wide but the deviations seem to be random variations rather than a tendency for the points to curve away from the line in any part of it. They are, therefore, attributed to errors in observation and particularly to the rather indefinite end point. The errors are largest at high temperatures where the time intervals for killing are very short and more difficult to measure accurately by the method used.

Since the method of fitting the curve is essentially a method of averaging all the observations, it seems reasonable to suppose that the curve or the equation of the curve represents the time necessary to kill the potatoes at any given temperature better than any single observation made at that temperature. They also fairly represent what may be expected at intermediate temperatures at which no observations were made.

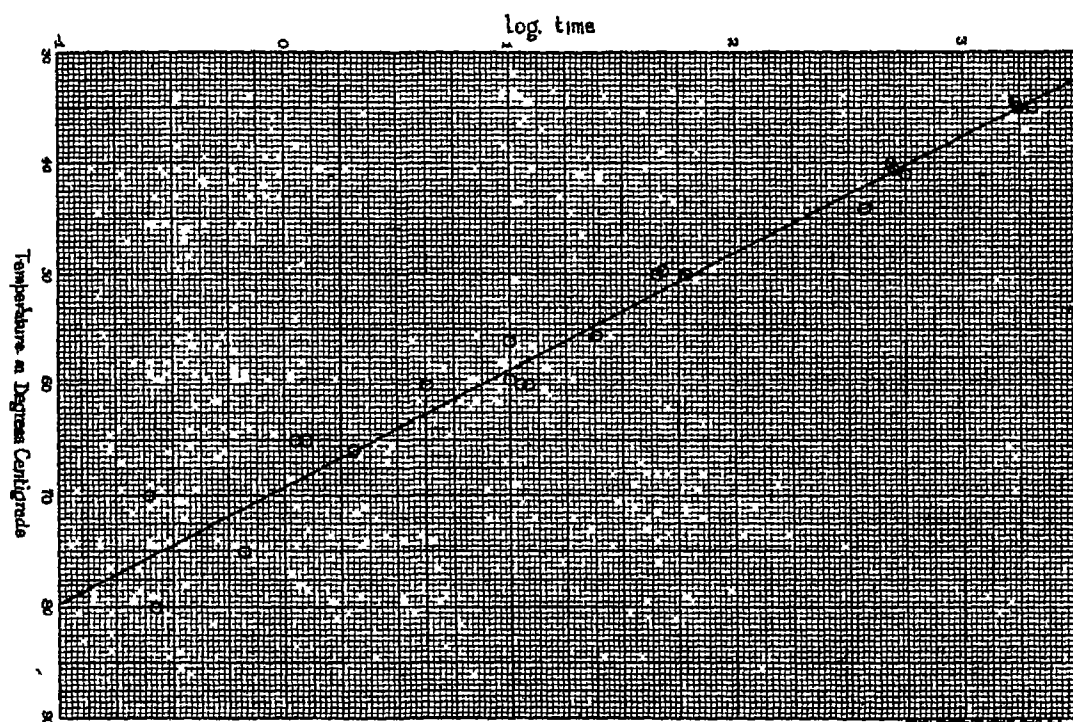


FIG. 1. Graph showing time (expressed as log time in minutes) required to kill potatoes when treated at different temperatures in hot water.

In the equation of this curve the constant m represents the slope of the curve or the amount of change in the logarithm of the time for one degree change in temperature. This would be equivalent to changing the time by multiplying it by the antilogarithm of m for each degree of lowering of the temperature, i. e., by 1.246, for a lowering of two degrees by $(1.246)^2$, for a lowering of 10 degrees by $(1.246)^{10}$ or 9.02.

To illustrate, it may be calculated from the equation that at 70° C. potatoes are killed in about .8 of a minute. If the temperature is lowered by 10° to 60° C. the time is $9.02 \times .8$ or 7.218 minutes. At 50° the time is 65.1 minutes and at 40° it is 58.72 minutes.

A similar relation was found by Miss Chick (3) between time and temperature in the killing of *B. Typhosus* by hot water. Bigelow (2)

has also found that this type of curve resulted when the thermal death times were determined for fifteen thermophilic bacteria. The writer has also found indications that similar results are to be expected in killing potatoes in mercuric chloride solutions, though the experiments so far made have not been very extensive.

So far as the work with dilute mercuric chloride solutions has progressed, it may be said that the time temperature curve for killing potatoes seems to be a little below the hot water curve and approximately parallel to it. This suggests that the hot water curve may serve as a rough guide in selecting treatments of suitable duration in other experimental treatments of potatoes with dilute disinfecting solutions.

It may also be mentioned, though no careful study was made, that in the treatments in hot water, potatoes treated in the neighborhood of the time-temperature combination necessary for killing, in general, exhibited symptoms of black heart. Not many of the potatoes were cut open as it was desired to see whether they sprouted without causing additional danger from rot by exposing cut surfaces. However, black heart was observed throughout the range of temperatures tried when treatment of suitable duration was made, even at temperatures up to 100° C. At the higher temperatures the range of duration of treatments in which black heart occurred was very limited. If the potatoes were treated too long at the higher temperature (60° to 100°) the tubers were apparently completely killed before the black heart had time to develop and in this case they remained white. Also at the higher temperatures a ring is formed, at short intervals just at the surface of the potato and at increasingly longer treatments the ring grows smaller and smaller until finally a dark spot only is formed at the center of the potato. Presumably the part outside the ring is killed so quickly that it remains white while the part inside has not been treated long enough to bring about this reaction.

TREATMENT OF POTATOES IN A HOT AIR OVEN

After most of the treatments of potatoes in hot water had been made, it was noted that the time necessary to kill potatoes in this manner was much shorter than the time necessary to kill potatoes in a hot air oven as reported by Pethybridge (7) and by Bartholomew (1) at temperatures where comparisons could be made. Therefore, it seemed desirable to make some treatments in a hot air oven for comparison. It appeared not impossible that the killing of the potato or of the virus of mosaic would be brought about by a sufficiently different reaction so

that the virus could be destroyed by this method without killing the potato even though the hot water method had failed.

The general methods used were similar to those used in the hot water treatments. The potatoes were suspended in a Fries hot air oven by cotton strings to avoid contact with metal. The air in the oven was kept in circulation by means of an electric fan. The potatoes used were mostly affected with mosaic, though a few treatments were made with healthy potatoes. The potatoes which sprouted after the treatments were planted in the greenhouse. All potatoes that were known to be affected with mosaic before treatment gave rise to mosaic progeny when planted. From the standpoint of the control of mosaic, then, this treatment is a failure.

By the same method as in the hot water treatment a summary of the killing of potatoes in a hot air oven was prepared. These results are given in table 3 and also shown in figure 2. In this case plotting the

TABLE 3—*Time required to kill potatoes (variety Magnum Bonum) in a well ventilated hot air oven at different temperatures.*

Temperature in degrees Centigrade	Time in minutes	Conditions of potatoes	Date
35	187200	Mosaic	3/30-9/11/22
40	6300	"	8/14/22
45	1980	"	3/23-24/22
50	270	Healthy	3/20/22
60	77.5	"	3/21/22
60	55.5	Mosaic	8/23/22
65	32.5	"	8/24/22
70	29	"	8/29/22
70	35	Healthy	3/20/22
70	32.5	Mosaic	3/22/22
80	19	"	3/25/22
90	20	"	3/28/22
100	11	"	4/7/22
120	10	"	3/29/22

logarithms of times as ordinates and the temperatures as abscissas does not give a straight line curve but proves to offer a convenient scale to work with where so wide a range of times are involved. Also a comparison may be made directly between the two treatments when they are both plotted in the same way.

A general expression was sought that would represent empirically at least the length of treatment necessary to kill potatoes throughout the range of temperatures at which tests were made. After numerous trials

of different curves, it was found that a curve represented by an equation of the form

$$(y - a)(x - c)^3 = b$$

could be made to fit the points as plotted as closely as could be reasonably expected considering the obvious irregularities in the observations. In the above equation x is temperature in degrees Centigrade and y is time of treatment in minutes necessary to kill potatoes. In determining the values of the constants, c was approximated by substituting pairs of values of x and y found experimentally and eliminating a and b between three such equations and solving for c . This was done several times with

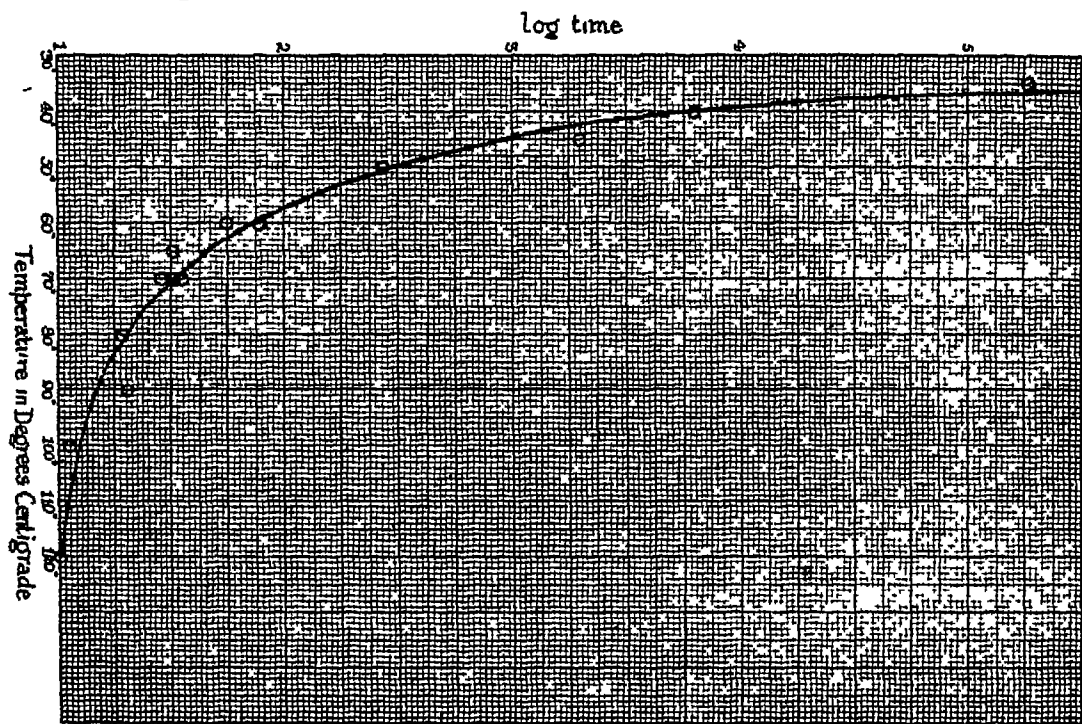


FIG. 2. Graph showing time (expressed as log time in minutes) required to kill potatoes when treated at different temperatures in a well ventilated hot air oven.

different series of pairs of values of x and y . The average value for c thus found was taken. Values were then found for a and b by the method of least squares. In this way the following values were found for the constants.

$$\begin{aligned} a &= 8.4255 \\ b &= 1057500 \\ c &= 34.5 \end{aligned}$$

The curve as drawn in figure 2 represents this equation. Since the eye can judge better as to whether a straight line fits a series of points the same results are plotted in figure 3 with $\log(y - 8.4255)$ as or-

dinates and $\log (\tau - 34.5)$ as abscissas. This curve is plotted with a slope of minus three and the fit of the observation points to this curve serves to confirm the exponent three of the quantity $(x - c)$ which was somewhat arbitrarily chosen. The observation points seem to fit this curve as well as might reasonably be expected except the point representing an observation at 35°C . The accuracy of this observation is indeed open to question for various reasons.

Probably too much significance should not be attached to the constants of an equation thus empirically found particularly when they apply at

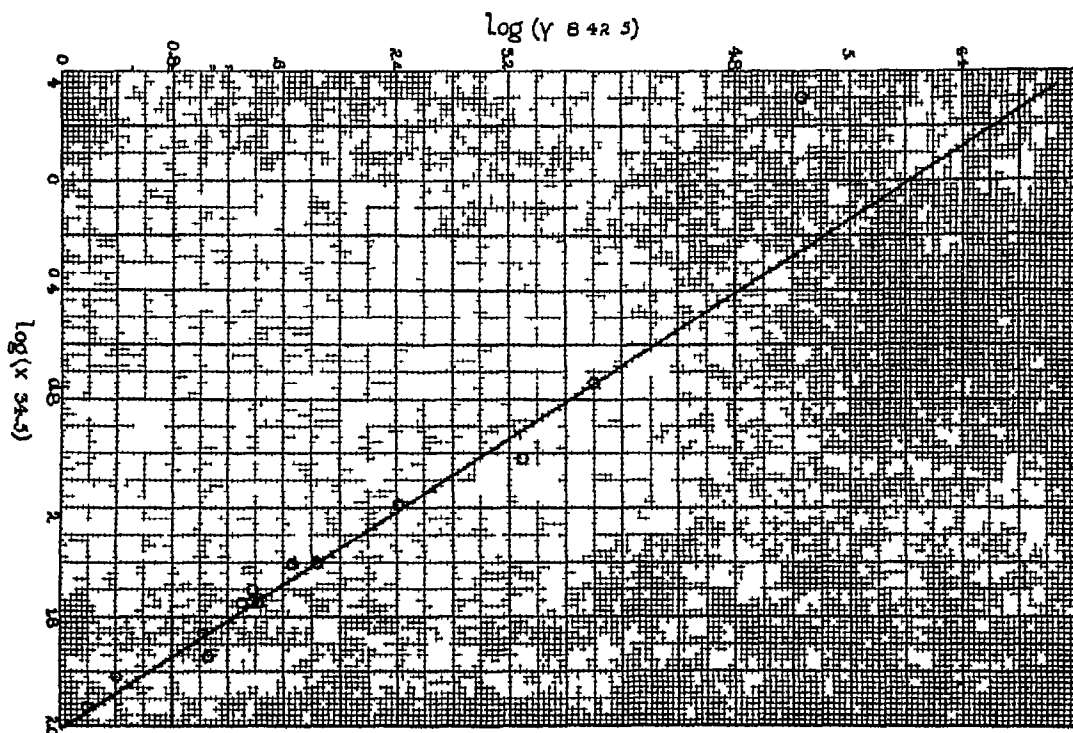


FIG. 3 To test further the fit of the equation $(y - 8425)(x - 34.5)^3 = 1057000$ it was changed to the form $\log (y - 8425) = \log 1057000 - 3 \log (x - 34.5)$. In this graph $\log (y - 8425)$ are plotted as ordinates and $\log (x - 34.5)$ as abscissas to show how closely a straight line is approximated.

temperatures outside the range in which tests were made as in the case of *a* or at the extreme end of the range as in the case of *b* and *c*. However this does not invalidate the use of the equation with the values found for the constants substituted in it for the purpose of calculating approximately the time necessary to kill potatoes by such treatments at any temperature within the range studied.

Furthermore it is evident that there must be a limit in temperature in the neighborhood of 35° below which potatoes will sprout and above

which they will be injured. From the form of the equation it is clear that the curve approaches the line $x = c$ asymptotically and thus c (found to equal 34.5) is an approximation of the limit of temperature in degrees Centigrade below which potatoes are not killed by such treatments.

In this connection the behavior of potatoes treated at 35° C. is interesting. When the potatoes were placed in the chamber at this temperature, they already had short sprouts about one half centimeter long. As long as they remained at this temperature no perceptible growth of sprouts occurred but as they were removed from time to time sprout growth was resumed. However, as the potatoes remained longer up to several months the old sprouts took on a yellowish color and resumption of sprouting when the potatoes were removed was slower and more feeble. When the last potato was removed after 130 days only two fine spindling sprouts (about .8 mm. in diameter and 1.5 cm. long) developed in the course of a month.

As to the other constants it is evident that b should be the time in minutes necessary to kill potatoes at $(1 + c)$ degrees Centigrade, i. e., at 35.5°. The value of b as found (1057500) does not agree with the one observation made at 35°.

The constant a (equals 8.4255) is time in minutes. The form of the equation would indicate that one could not expect to kill potatoes in shorter periods than this even by raising the temperature of the oven. This could hardly be accepted without further experiments.

It is believed, however, that this equation may be taken as representing approximately the relation between time and temperature necessary to kill potatoes in a hot air oven within the range of temperatures at which tests were made (35° to 120° C.). It is hoped that as in the case of the treatment of potatoes in hot water it will indicate the limit beyond which it is unsafe to go in treating potatoes for any parasitic disease by this method. In fact, it can not be approached very closely without serious injury to the potatoes.

This curve may also be taken as an indication of conditions under which injury may be expected to potatoes in storage or transit. However, it is probable that the times represented on this curve apply only to well ventilated potatoes as the ventilators were kept open on the oven used. It has been shown by Stewart and Mix (9) that lack of air brings about a similar injury at low temperatures. Such heat injury to potatoes is apparently not uncommon in certain places. In a report by Mann and Nagpurkar (6), they state that when potatoes were stored in a well aerated room, in a place where the shade temperature went up

to cover 100° F. in a few days, a large part of the stock became rotten and useless. They also state that there is no danger in storing potatoes below 86° and little danger below 90° F. General rotting has also been reported at times in potatoes transported in the holds of ships, which seems to merit investigations along these lines.

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EARLY STAGES OF CROWN GALL

MELVILLE T. COOK

WITH FOURTEEN FIGURES IN THE TEXT

The study of plant galls due to various causes has resulted in the accumulation of much interesting data but very naturally many points have been overlooked. In general it may be said that plant galls follow the same general lines of development regardless of cause of formation. This very general statement may be analysed as follows:—(1) The excitation or stimulant must be applied to the meristematic tissue. In the case of some galls caused by insects, the experimental work indicates that the stimulant must be applied to the primary meristem or cambium. In the case of crown gall it has been claimed that the tissues other than the cambium were susceptible to stimulation but these statements are far from being definite. (2) The first result of this stimulation is the formation of a homogeneous parenchyma tissue instead of the normal differentiation of tissues which is characteristic of the normal organ. In some cases this homogeneous tissue is characteristic of the various species of galls. (3) It is doubtful if any tissue, having become fixed, can be stimulated into activity; but it is evident that some tissues, which under normal conditions are fixed, may be stimulated into activity. (4) It has been demonstrated in a large number of cases that the cell activity may be in cells which are very remote from the organism and is due to some secretion which passes from cell to cell or to mechanical pressure resulting from the growth of neighboring cells.

This study of the crown gall was undertaken to determine the incipient stages of formation. However, some other data were incidentally acquired. The material used was castor oil bean seedlings about four inches in height and young plants of *Bryophyllum*, although most of the studies were made on the former. The inoculations were made into slits just below the cotyledons. Longitudinal slits were used instead of needle punctures in order to make sure that the organisms came in contact with all tissues from epidermis to central parenchyma pith. These slits were filled with the organism from cultures.¹ In making inoculations with a needle it is very evident that some of the organism may not penetrate to the depth of the needle point. However, a slit is a much more severe wound and probably causes a much greater shock

¹ The original culture was furnished me by Dr. Erwin F. Smith of the U. S. Bureau of Plant Industry.

to the growing plant tissues than a needle prick. In order to determine just how much of the reaction was due to the organism and how much was due to the wound, plants of approximately the same age were cut in the same manner with a clean, sterilized scalpel and held as checks. Of course it was impossible to make the slits exactly the same; some of the slits would pass through the fibro-vascular bundles while others would follow the medullary rays. At intervals of two days the slit regions of both the inoculated and check seedlings were cut into small pieces, fixed in weak Flemming fluid, embedded, cut and stained with haematoxylin-iron alum and safranin-gentian violet. Both the inoculated and check material were studied in comparison with tissues from an uninjured plant.

There is nothing unusual in the structure of the normal seedling castor bean. It is that of the ordinary herbaceous, dicotyledonous plant. It may be briefly described as follows:—epidermis with thin cuticle, a cortex consisting of about 12 or 15 layers of parenchyma cells which become larger in passing inward but which show little or no signs of collenchyma formation; distinct phloem, cambium, xylem, and pith; each consisting of the characteristic cell structures (figures 2 and 4).

The reaction to the wound may be described as follows:—an increase in the number of layers of cortex cells in the vicinity of the wound, thus forming the callus (Fig. 1, ca); sometimes a slight stimulation of the phloem dependent on the location of the injury; sometimes an increase in thickness of the cambium; a distortion of the xylem due to the growth of other tissues when the knife passes through a young fibro-vascular bundle (Fig. 5); a growth of medullary ray and pith parenchyma to form rows of cells (Fig. 3). Of course since reactions of this kind occur in uninoculated plants they cannot be attributed to the organism in the case of those plants which were inoculated.

In the case of the inoculated plants we find exactly the same reactions as in wounded plants, before we find any reaction which the writer was satisfied in attributing to the organism. Reasoning from our knowledge of other plant galls it was natural to expect the most pronounced reactions would be in the cambium, but such was not the case. The first reaction observed which the writer felt justified in attributing to the organism was in the cortex, or outer part of the medullary ray, i.e., between the phloems of two adjacent bundles. It was in close contact with the cambium (Fig. 6), and slightly removed from the point of inoculation. This was a mass of cells which the author will designate as a "whorl." It has been previously designated as a "whorl" and as a "nest" by Dr. Erwin F. Smith. It consists of cells which were richer

in protoplasm than the surrounding cells and more of them showed nuclei although none were multi-nucleate. They showed indications of growth in that some of them were larger and some smaller than the surrounding normal cells, the larger ones apparently having been stimulated into excessive growth and the smaller ones into excessive cell division. Although the whorl was clearly evident, the cells graded into the surrounding cells, so that it was difficult to determine an exact outline. Most of the cells, and sometimes all that are visible in a section, undergo repeated divisions, so that in many sections all the cells of a "whorl" are small, rich in protoplasm and each provided with a single nucleus (Fig. 7). In longitudinal section, these "whorls" have the same appearance, so that in reality they are very nearly spherical masses. The crown gall appears to be made up of one or more of these "whorls" or masses. It was at first supposed that these masses were restricted to the cortex but they were later found in considerable abundance in the pith. However, it appears from these studies that the cortex, which is in close contact with the cambium, is most favorable for the development of these "whorls."

The tracheary tubes of the fibro-vascular bundles were frequently pushed out of place by the mechanical pressure exerted by the "whorls," but there was no evidence to indicate that they were ever directly influenced or modified by the organism (Fig. 5). However, tracheids were very generally formed in the whorls and apparently directly from the parenchyma cells (Fig. 8). In some cases the nucleus and some protoplasm were still visible in these tracheid cells (Fig. 9). Instead of being arranged in more or less straight, perpendicular lines as in normal stems, they were placed in a very irregular manner. Later in the development of these "whorls," spiral tracheary tubes were formed. They also were apparently formed *de novo* from the homogeneous parenchyma of the "whorls" and not from the fibro-vascular bundles.

The strands of cancer tissue which have been described by Smith were very easily observed in both pith and cortex. Those in the pith were of greater diameter than those in the cortex; the distal end was blunt and resembled the whorl structure previously referred to (Fig. 10). The result of pressure on the surrounding cells was very evident, especially on those cells lying between the strands and the fibro-vascular bundles as shown by the formation of tracheids (Fig. 10). The strands in the cortex appeared to be formed from both cortical and cambium cells, and in every case observed, were between the phloem of two adjacent bundles (Fig. 11). The direction of growth was both up and down the stem. From time to time these strands push outward,

forming cone shaped masses of cells which finally break through the cortex and epidermis (Figs. 12-14). There is none of the "whorl"-like structure previously referred to but the cells are small. At first, this cone shaped body appears to be composed entirely of cells formed from the cortex and cambium but a little later, the pith (or probably more properly the medullary ray tissue) pushes or grows up from below and forms the center of the new or secondary gall (Figs. 13 and 14). Before the tip of the new gall has broken through the epidermis, tracheids are being formed in the base of the gall, apparently from the pith parenchyma cells (Fig. 14).

The galls very soon reach a degree of complexity in which the distortions are so great that it is sometimes impossible to tell just which tissues are of abnormal development and just which ones have been pushed out of place by the growth of others.

The sections showed the organism in abundance in the original slits and sometimes in small pockets connected with the slits but the writer was unable to trace the organism to the "whorl" or strands.

The studies show that the primary meristem or cambium does not respond to stimulation from the organism so quickly as in the case of many other galls. It is furthermore apparent that all meristematic cells may respond to stimulation from the organism. The question naturally arises as to when the cells cease to be meristematic. We will not attempt to answer this question, other than to say that the tracheary tissues have apparently ceased to be meristematic and that they are influenced only in so far as they may be pushed out of their normal position by the pressure exerted by the growth of neighboring tissues.

The cells of the incipient gall are parenchyma, are rich in protoplasm and are uni-nucleate. The only noticeable variation was in size of the individual cells. The tracheary tissues arise from these parenchyma cells.

The young shoots of *Bryophyllum* have a much weaker fibro-vascular bundle than the seedling plants of castor oil bean, the cortex is somewhat thicker and the pith more prominent. The response to a slit wound is very similar; the cells of the cortex and pith tend to arrange themselves in rows but the distortion of the fibro-vascular bundle is much more prominent. In the case of those plants which were inoculated the whorls were formed in practically the same manner as in the castor bean but in some cases the connection with the cambium was much more pronounced.

In order to determine the question of response of tissue to the organism, fourteen nodes of a growing shoot were inoculated beginning with the outermost. Well defined galls developed at the six outermost

nodes. Sections through the other nodes showed evidence of cellular disturbances which would probably have developed into galls at a later date.

SUMMARY

The stimulation is not restricted to the cambium.

Parenchyma tissue is especially susceptible to stimulation.

The galls start as more or less spherical masses in the parenchyma cells which are stimulated into activity. The growth is different from the callus growth following wounds.

The organism evidently exerts considerable influence in cells with which it is not in contact.

The xylem of the fibro-vascular bundle may be distorted as a result of a wound or as a result of pressure exerted by growing gall tissues but it is doubtful if it is otherwise modified.

Tracheids and spiral tubes appear in the galls but are evidently developed *de novo* from homogeneous parenchyma mass of the incipient gall.

Tumor strands may develop in the pith or in the cortex in close contact with (or possibly involving) the cambium. These latter strands lie between the phloem of the two adjacent fibro-vascular bundles.

The author wishes to express his thanks to the New York Botanical Garden for facilities and use of the library in conducting these studies and to members of the staff for assistance in many ways.

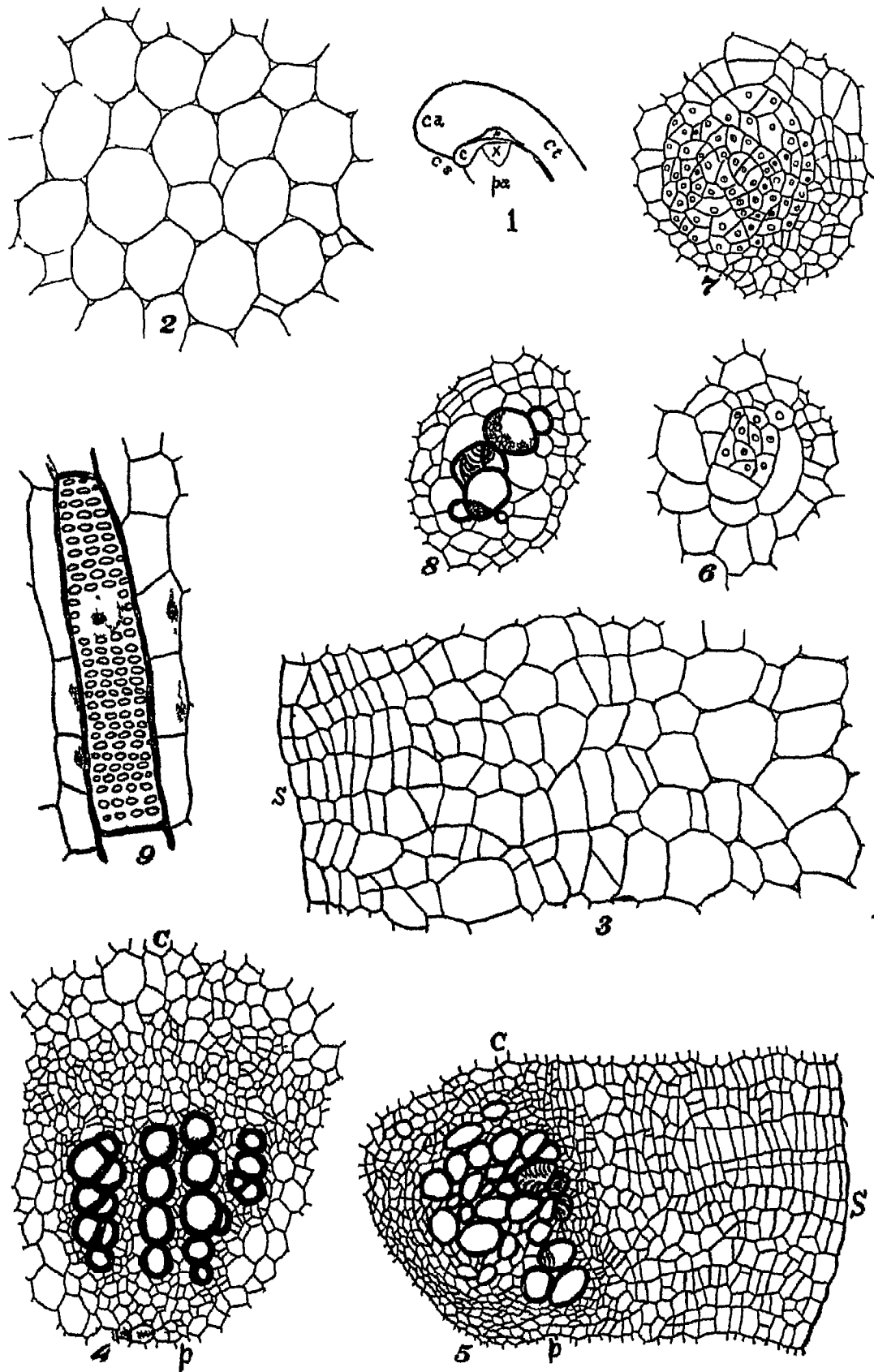
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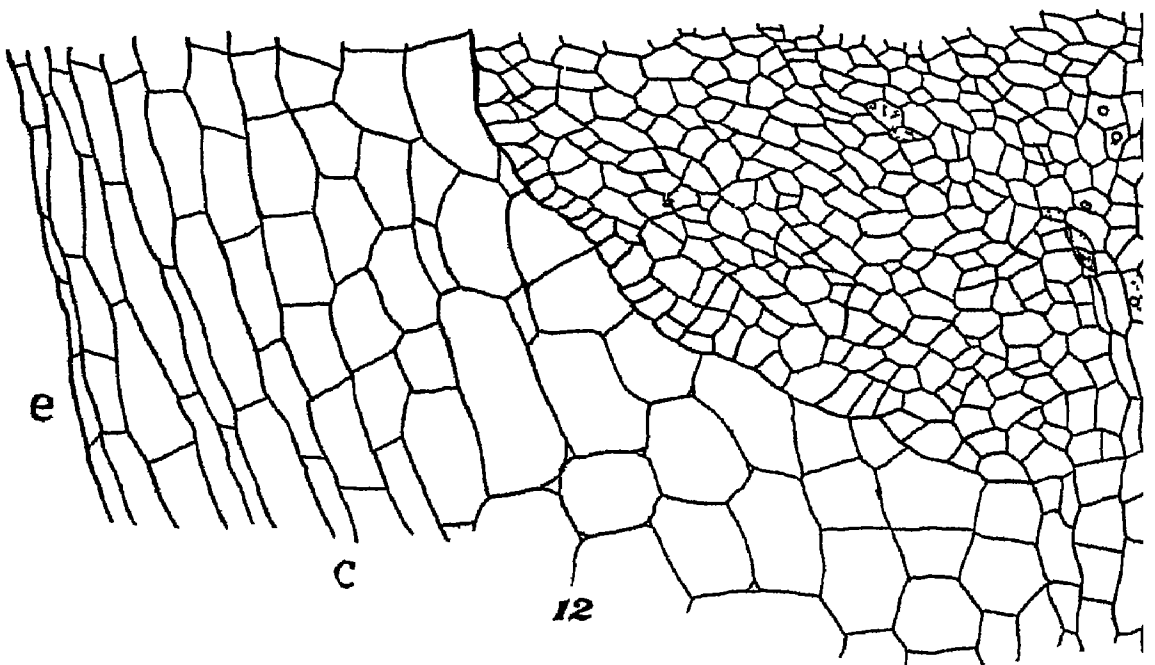
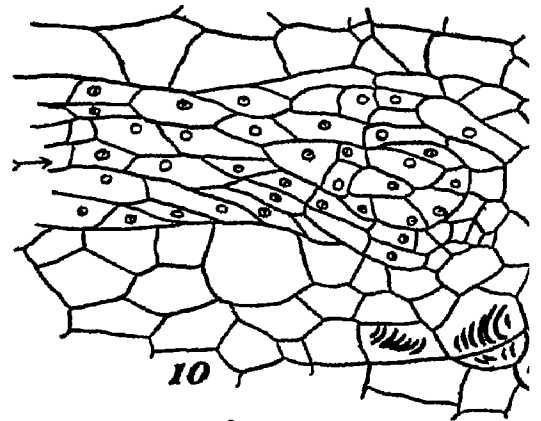
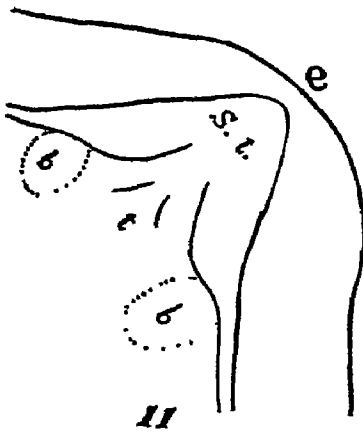
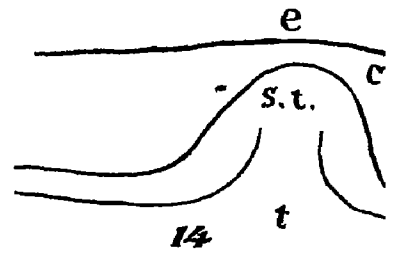
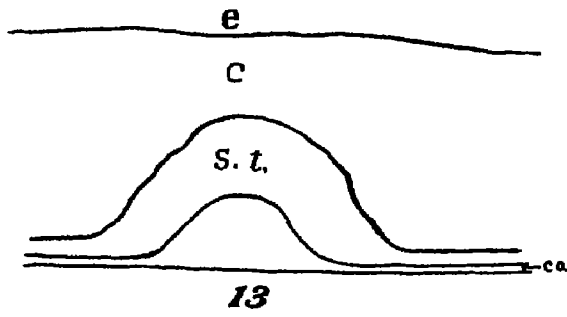
RIO PIEDRAS, PORTO RICO.

EXPLANATION OF FIGURES

All drawings were made from sections of castor bean seedlings.

Fig. 1. Diagrammatical drawing of part of cross section. c.s.—cut surface; pa—parenchyma of pith; c—cambium; x—xylem; ct—cortex; ca—callus. Fig. 2. Normal parenchyma cells from pith. Fig. 3. Cross section of pith parenchyma showing tendency for arrangement of cells in rows following a wound. s—cut surface. Fig. 4. Cross section of normal fibro-vascular bundle. c—cortex side; p—pith side. Fig. 5. Cross section of fibro-vascular bundle showing distortion. s—cut surface through medullary ray; c—cortex side; p—pith side. Fig. 6. Young "whorl" in cortex, in close contact with cambium, and between the phloem of two adjacent bundles. Fig. 7. Older "whorl" in cortex, in close contact with cambium. Fig. 8. "Whorl" showing the formation of tracheary tissue. Fig. 9. Tracheid cell in which the nucleus is still visible. Fig. 10. The tumor strand in the pith, showing the advancing tip. Also showing the formation of tracheary cells apparently as a result of mechanical pressure. Fig. 11. Diagrammatical cross section drawing showing position of secondary tumor arising from cortex intermediary between two fibro-vascular bundles. s.t.—secondary tumor; e—epidermis; f—fibro-vascular bundle; t—early formation of tracheary cells. Fig. 12. Drawing of part of section of secondary tumor apparently arising from the cambium; ca—cambium; c—cortex; e—epidermis. Fig. 13. Diagrammatical longitudinal drawing of secondary tumor. s.t.—secondary tumor; c—cortex; e—epidermis; ca—cambium. Fig. 14. Diagrammatical longitudinal drawing of slightly older secondary tumor. s.t.—secondary tumor; c—cortex; e—epidermis; t—region of early formation of tracheary tissue.





HELMINTHOSPORIUM HEVEAE IN SUMATRA

CARL D. LA RUE

Helminthosporium Heveae, Petch, a parasite of the Para rubber tree, *Hevea brasiliensis*, has been known in Ceylon, where it was described by Petch, for about fifteen years. As late as 1911 Bancroft¹ stated that there was as yet no record of the occurrence of this disease in the Federated Malay States. The writer has not seen any note of its occurrence in the Federated Malay States since that time, but according to Butler² the disease occurs in Malaya, as well as in Ceylon, South India, and Java. There is no record of this disease in Sumatra although it has doubtless existed there for some time. The observations of the writer are confined to the years 1917-1920, but the fact that the disease was already widely spread in 1917 indicated that it had been present for at least a few years previous to that time.

Both Petch³ and Butler state that the disease is confined to young trees, that it does not cause defoliation, and that the damage is not severe.

Observations in Sumatra made in 1917 and 1918 by the author are quite in agreement with those of Petch and Butler. Spotting of leaves from the disease was seen to be very common in nurseries and in estates just planted. In some cases the spotting and malformation of the leaves have been the cause of considerable loss of vigor in the seedlings. After the first wintering, however, usually no traces of the disease could be found.

In 1919, on the contrary, the disease was found on old trees on several different estates; on one area several hundred trees were affected. The severity of the disease varied greatly on different areas and with different individual trees; on some it was confined to a few spots on some of the leaves, while in the worst cases almost every leaf was riddled with spots. Malformation of the leaves was commonly seen but was not pronounced in extent. The attack was especially noticeable just as the young leaves were coming out. Some of the affected trees were partly defoliated,

¹ Bancroft, Keith. The Dieback Disease of Para Rubber and a note on the Leaf Disease of Para Rubber. Fed. Malay States Department of Agriculture. Bull. 14.

² Butler, W. J. Fungi and Disease in Plants. Thacker, Spink & Co. Calcutta & Simla. 1918.

³ Petch, T. The physiology and Diseases of *Hevea Brasiliensis*. Dulau & Co., Ltd., London, 1911.

but a careful examination indicated that the defoliation was due to the attack of mites rather than to the activity of the fungus.

INFECTION

Infection appears to take place in most cases while the leaves are still very small and not yet unfolded. With the growth of the leaves to a larger size still more spots are formed, but apparently almost no infection occurs after the leaves have reached full size and have become cutinized on the surface. Spotting occurs on the petioles and the mid-ribs as well as on the laminae of the leaves. Spots on the bark of the twig are comparatively rare. The number of spots on a leaf varies from one or two to some dozens; in the worst cases one half or more of the assimilating area of the leaf is invaded by the fungus and killed or rendered useless. Where all or most of the leaves of a tree are so attacked the disease may be considered serious because of the interference with the food supply of the tree.

Evidence is lacking that the disease affects parts of the tree other than the leaf-blades and petioles, and occasionally the bark of the young twigs. Cultures of the tips of twigs, which bore infected leaves but which were not themselves spotted, did not show *Helminthosporium*, so it appears that the fungus does not enter the twigs from the leaves. Likewise it appears that the leaves are not infected by growth of the fungus from the twigs but by wind-borne spores.

The source of spores in this case was probably the leaves of the previous season which were seen to be more or less spotted. Given proper conditions for infection these old leaves could easily infect the new ones. It is not known whether the fallen leaves disseminate spores or not but the difference in time of wintering would allow a few diseased trees which winter late to infect a large number of trees just as the leaves unfolded.

It is probable that nurseries and estates are sometimes infected from crops planted by natives in their villages. The question of the susceptibility of the various common crops to *Helminthosporium* attack has not been investigated, but the writer has very often seen what appear to be *Helminthosporium* spots on *Manihot* and papaya. It is probable that the disease is harbored by several common plants, from which it can spread to rubber nurseries and plantations.

GROWTH OF THE FUNGUS IN CULTURE

The organism has been grown in culture by the author and has been found very easy of cultivation. On agar made with a decoction of

Hevea leaves and twigs it grows very well. It also grows satisfactorily on bouillion agar. Fruiting does not take place on either of these media until the culture is quite old and the agar rather dry. No special attempts were made to induce more rapid fruiting but it is likely that this could be done.

The mycelium of the fungus is at first white but soon turns dark on the surface of the agar, and after a short time nearly all the mycelium is dark in color with a white fringe around the outer edge.

The fungus when grown on raw potatoes failed to show active parasitism. A slight dry rot over a small area was the only development.

SIZE AND CHARACTER OF THE SPORES

The spores of the fungus when first formed are hyaline, curved, and non-septate. Later they darken and become vacuolate with several large vacuoles. Finally they are quite dark with rather evenly spaced septa. Sometimes the vacuolate condition of the spore persists, but whether or not the spores which remain in the vacuolate condition are viable is unknown. The description of the spots and spores of *Helminthosporium*, as given by Petch, fits the fungus in Sumatra very well except that the spores of the Sumatran form appear to be much smaller, especially when produced in culture. Petch gives the spore length as 100 to 200 microns while from 100 measurements of spores from one culture the following distribution of lengths was secured by the writer:

<i>Spore lengths in microns</i>	<i>Number of spores</i>
32.8	4
36.6	1
30.4	7
34.2	8
38.0	7
41.8	16
45.8	14
49.4	9
53.2	8
57.0	6
60.5	5
64.4	4
68.4	2
72.2	3
76.0	3
79.8	2
83.6	1
Range of spore length	22.8-83.6 microns
Mode of distribution curve	41.8 microns
Mean spore length	47.44 microns

Thus the largest spore measured from this culture is smaller than the smallest length given by Petch.

Eighty measurements of spores taken from a leaf of *Hevea* gave the following frequency distribution.

<i>Spore lengths in microns</i>	<i>Number of spores</i>
38.0	1
45.6	3
53.2	2
60.6	6
68.4	4
76.0	4
83.6	14
91.2	17
98.8	12
106.4	10
114.0	7

The spores grown on the host plants are much larger than those grown in the culture which was measured but whether the difference is due to cultural conditions or to difference in strains is not known. The former is possible as the culture was isolated more than a year before the spores from the *Hevea* leaf were measured. It is equally possible that the two sets of measurements represent distinct strains and that other strains may exist which are as large as those measured by Petch.

La Rue and Bartlett¹ were able to distinguish a number of strains in *Pestalozzia Guepini* Desm. and the multi-lineate condition of such organisms as the wheat rusts is too well known to need mention. The same condition doubtless exists in many other species of fungi not yet critically studied and *H. Heveae* may well be one of this number.

It is possible also that changes in environmental conditions from one year to the next may cause changes in spore size.

Petch gives the width of the spore of the fungus as 15–18 microns. Measurements by the author of width of 25 spores from a leaf of *Hevea* gave:

<i>Width in microns</i>	<i>Number of spores</i>
11.9	3
13.6	11
15.3	9
17.0	2
Mean spore width	14.28 microns
Range of width of spore	11.9–17.0 microns

¹La Rue, Carl D. and H. H. Bartlett. A demonstration of numerous distinct strains within the nominal species *Pestalozzia Guepini* Desm. Amer. Jour. Bot. 9: 79–92. 1922.

These measurements agree fairly well with those of Petch although Petch does not report any spores so narrow as some found here.

The spores from the Hevea leaf which were measured for length were also examined for number of septa. The distribution is as follows:

<i>Number of septa</i>	<i>Number of spores</i>
1	1
2	0
3	3
4	4
5	3
6	9
7	24
8	19
9	14
10	3
Mean number of septa	7.12

These figures are also close to those given by Petch, which are 8–11 septa. The range here, 1–10 septa, is much greater than that given by Petch but it is possible that he examined only the larger spores without taking a random sample. This may possibly have been the case with all his measurements while those of the writer were all made on spores taken at random.

SUMMARY

Helminthosporium Heveae Petch is more or less widespread in the whole of the eastern rubber plantation area. It has been known in Ceylon and the Malay Peninsula, and its range is now shown to include Sumatra where it occurs on the East Coast. It usually infests nurseries where Hevea trees are planted but in 1919 it attacked mature trees also. The spores of the fungus in Sumatra appear to be smaller than those measured by Petch in Ceylon.

ANOTHER ECONOMIC HOST OF BACTERIUM SOLANACEARUM

COLIN G. WELLES AND EMILIANO F. ROLDAN

WITH TWO FIGURES IN THE TEXT

During the month of February, 1921, a serious wilting of mature tañigo plants (*Chrysanthemum coronarium* Linn.)¹ occurred in the gardens of the College of Agriculture, Los Baños, Philippine Islands. The disease persisted until May 1, when all of the plants were killed or so seriously affected that death was imminent.

The senior writer, while in China, learned that *C. coronarium* is used daily by most Chinese families from Canton as far north as Shanghai. The Cantonese name given to the plant is *tung-hao*. From the Chinese name the Tagalogs have derived the local name *tañigo*.

The college gardens have been known to be infested with *Bact. solanacearum* E. F. S. for several years, and because of this fact the wilt of tañigo was believed to be caused by the above named organism. On this hypothesis the following work was undertaken.

In a brief note the writers (1) have given, previously, a description of the disease and have stated that it was believed that *Bact. solanacearum* was the cause.

SYMPTOMS

The wilt of tañigo appears very similar to wilt of solanaceous plants caused by *Bact. solanacearum*. The seedlings are highly susceptible and are entirely destroyed within three days after the first appearance of the wilt, particularly in the dry season. The older leaves are diseased first and shortly thereafter the younger leaves become prostrate.

¹ *Chrysanthemum coronarium* Linn. is a plant of Mediterranean origin. Dean Charles Fuller Baker of the College of Agriculture, Los Baños, has very kindly offered the following information concerning the cultivation of this plant. To his knowledge it is cultivated in Cuba, and other islands of the West Indies, Philippine Islands, Straits Settlements, Borneo, and all other South Sea Islands which he has visited.

The following is taken from *The Commercial Products of India* by Sir George Watt: "This cultivated little herb is of interest mainly because of the fact that from Dinajpur and Rangpur in Bengal, north-east to Assam, it is extensively eaten, and is number 15 in Buchanan-Hamilton's list of saga or pot-herbs Plants of the pea family become scarce but . . . a peculiar series of pot-herbs take their places. Among these *C. coronarium* was found to hold a foremost position, being known as *babir* in North Bengal, *babui* in Assam, and *jalyniar* in the Khasia hills. Speaking of Formosa, Walters calls it *tan-ei-tsai* and adds that it is a 'herb much used by the Chinese as an article of food.' Bretschneider makes mention of two species of *Chrysanthemum* as known to the Chinese classics, the leaves of one of which are boiled into soup."

The older plants while very susceptible to wilt resist for several days before the leaves wilt and die. These older plants have thick woody stems which support the wilted dry leaves until intentionally destroyed.

ISOLATION OF THE ORGANISM

The vascular elements and to some extent the parenchymatous tissue in severe attacks appear packed with motile bacteria.



FIG 1 Healthy and wilted taño plants. The plant to the left was inoculated through needle pricks in the stem about one week previous to the taking of the photograph. Both plants were held in the damp chamber for the entire period. The plants are half grown.

Isolations were made from sections of diseased stems and petioles and a white organism was obtained in nearly pure culture. Transfers were made to nutrient and potato agar and from these, inoculations were made into taño seedlings.

INOCULATIONS

Seedlings of taño when inoculated with a young culture of the isolated organism by needle pricks in the stems wilted after varying periods depending on the age of the plant and its treatment after inoculation. The time of incubation fell between ten and twenty days. The periods

were shorter when the plants were held in a moist chamber and longer when inoculated in the field. When older plants were inoculated, wilt never developed unless the plants were kept in a damp chamber.

In each case where the plants were diseased the bundles of the stem were examined and were found always to be teeming with bacteria. In several cases re-isolations were made and the original type of organism was obtained.

Authentic cultures of *Bact. solanacearum* taken from tomato, egg-plant and castor bean were available and tomato seedlings, not more

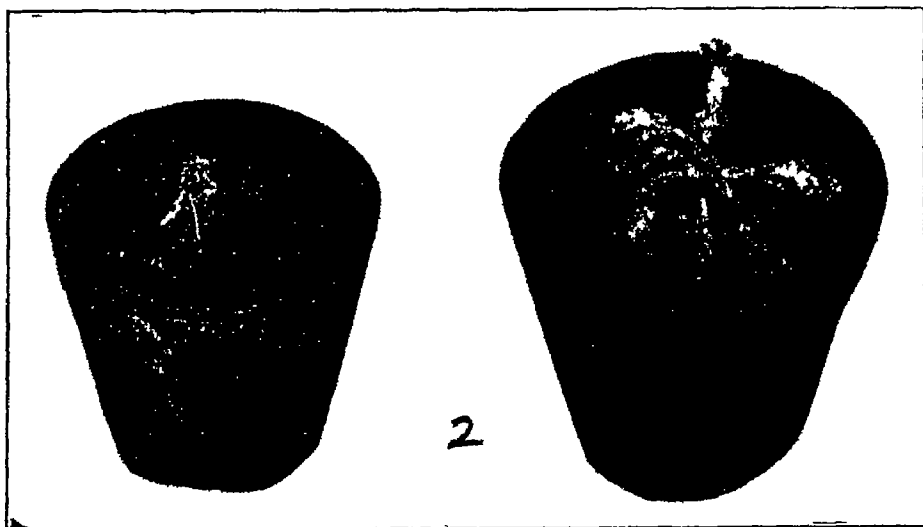


FIG. 2. Wilted and healthy taño seedlings. The plant on the left was inoculated through needle pricks in the stem with the taño organism about one week previous to the taking of the photograph. The plants were kept out of doors for the entire period.

than 10 centimeters in height, were inoculated with them as well as with a culture of the taño organism. In each instance the plants were inoculated through needle pricks and held in the damp chamber until the disease appeared.

After four days three seedlings inoculated with each organism wilted, and after six days all inoculated plants succumbed with the exception of one which had been inoculated with the tomato and another which had been inoculated with the egg-plant strain. The relative pathogenicity of each organism appeared to be about the same on this host.

Smith and other workers have frequently described the growth of adventitious roots near the point of inoculation, particularly on tomatoes and nasturtium. This phenomenon has never been observed locally, but instead a more or less brownish lesion is formed at the point of inoculation which at first was believed to be caused by organisms other

than *Bact. solanacearum*. However, many inoculations have been made after washing the stem thoroughly with mercuric chloride and sterile distilled water and the lesion invariably appears.

The vascular tissue in the young plants inoculated with the taño organism turns brown and is identical with the vascular reaction of the solanaceous hosts.

CAUSAL ORGANISM

The organism isolated from taño is fundamentally similar to the organism isolated from tobacco, tomato, egg-plant, and castor bean and to the organisms used by Erwin F. Smith (2, 3) in cultural studies. The one consistent variation is in that of pigment production. When isolated from taño the organism produces but a very slight brownish coloration and that pigment which is formed is confined wholly to the bacterial growth.¹

After the organism is passed through tomatoes the pigment producing power is regained but the color from the organism taken from taño directly, whether originally isolated from taño or tomato, never forms the deeper brown pigment.

The organism from taño has been grown in most of the differential media and complies with the published records of Smith (2, 3) giving the following cultural number, 2—3333823.

MORPHOLOGY OF THE ORGANISM

The organism is very similar morphologically to the one described by Smith (2, 3). The rods are short, with rounded ends, measuring from .66 to 1.77 microns in length by .22 to .55 microns in width, the average measuring 1.22 by .42 microns. The organism stains readily with the common aniline dyes such as carbol fuchsin and methylene blue and shows a slight concentration of the stain at the poles.

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LOS BAÑOS, P. I.

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- (3) ———. Bacteria in relation to plant disease 3: 174-219. 1914. Carnegie Institution, Washington, D. C.

¹During the second season of observation, the organism isolated from wilted *C. coronarium* frequently, but not invariably, produced the typical dark brown pigment of the organisms isolated from the solanaceous hosts.

A BRIEF HISTORY OF CITRUS SCAB IN JAPAN

TYÔZABURÔ TANAKA

Citrus scab is regarded by Japanese pathologists as a disease known in Japan from ancient times. Nishida,¹ referring to a letter of T. Tamura, states that the disease has been known on Kunembo (a mandarin closely allied to King orange), Satsuma and other kinds of oranges as a pathological ulcer-scab called Kahi and he refers to local Japanese names Abata Mikan (small-pox orange), Kasakaki Mikan and Kasa Mikan (both meaning syphilis-scab orange).² Ideta³ also mentions that the disease was known in Japan from a long time past by the names Abata Mikan and Kasakaki Mikan. According to Hori,⁴ the disease is the commonest of all citrus diseases in Japan and the growers of Kunembo and Tengu Mikan (a tangelo, probably a natural hybrid) have thought the scabbiness to be the characteristic of these oranges. Hori also states that the origin of the disease is unknown as we lack the record, but he suggests its probable origin from China with the introduction of nursery plants of citrus. Nishida first identified this Japanese disease as that called citrus scab in America and he gave the Japanese name "Sôkabyô," written originally in Chinese characters meaning syphilis scab disease, which is now generally recognized as the standard Japanese term for the disease. As was pointed out by the writer,⁵ this paper of Nishida badly mixes up citrus canker and citrus

¹ Nishida, Tôji. Kankitsu no Shippei (Diseases of citrus fruits) in Kankitsu (Citrus fruits) 1 no. 3:3-4. M. 36, vi June, 1903. (Japanese). Also in Kankitsu Byôgaihen (A manual of citrus diseases) Shidzuoka, M. 40, v, May, 1907, p. 20-21. (Japanese). & Nishida's Shinpen Kankitsu Byôgai to Yobôhō (New discourse on citrus diseases and their prevention) Tokyo, T. 3, xi, Nov., 1914, p. 40-41. (Japanese.)

² There are no Chinese characters corresponding to these words, and they are all written in phonetic syllabaries of *Kana*.

³ Ideta, Arata. Zôtei Nippon Shokubutsu Byôrigaku (Handbook of plant diseases of Japan) 4 ed. Tôkyô Shôkwabô, M. 44, 1911. p. 737-738. (Japanese.)

⁴ Hori, Shôtarô. Kankitsu Sôkabyôshi (History of citrus scab) in Hori's Shokubutsu Byogai Kôwa (Lectures of plant diseases) Tôkyô, Seibidô, T. 5, 1916, p. 280-289. (Japanese.)

⁵ Nishida, Tôji. Kankitsu no Sôkabyô (Citrus scab) in Dainippon Nôkwaihō (Journ. Agr. Soc., Japan) no. 258: 1-4. M. 36, ii, Feb. 1903. (Japanese.)

⁶ Tanaka, Tyôzaburô. A brief history of the discovery of citrus canker in Japan and experiments in its control (Quarterly Bull. State Pl. Board of Florida) 3 vol. 1. 1918, p. 3.

scab, but the former was later separated by the name "Marugata Sôkabyô" (Round scab) or, more definitely by the name "Kankitsu Kwaiyôbyô" (Canker of citrus).

The earliest occurrence of the Japanese name of citrus scab in the literature is probably in the work mentioned by the writer,¹ as Nankai Hôfu (Oranges of South sea district) written by Yoshiyuki Murase in 1818.² There are two scabby oranges mentioned in this work, one under the Chinese name "Shitôkan" (Lion-head orange) and the other "Reishikitsu" (Lychee orange), of which the second one bears the Japanese name "Kasa Mikan" in *Kana*. Very fortunately, the text of the description of Shitôkan was published by Ohara (see note 8), and it is the only description selected from the original manuscript work and printed. The comparison of this text and a figure attached to it, with those of the revised manuscript copy, shows that they are not exactly the same, and certain characteristics of the orange mentioned in the former are omitted in the latter. The page of text printed by Ohara, treating the Shitôkan or Lion-head orange, says "the size of fruit is large, about one foot in girth, two-tenth of a foot high,³ with "Hirui"

¹ l. c. p. l.

² There is a bibliographic error in my citation of this work (7), which gives the name of the author as Yamanaka Shinko and the date of preface as 1865. The manuscript copy the writer has, gives S. Yamanaka as the author but this is an error. He merely made additions to the original text. The other copy in the Library of the Department of Agriculture (93 N 152) originally gave Yamanaka as compiler, but this was afterwards changed to read that he enlarged and corrected the work and Tôdô Ohara, is added erroneously as the original author. According to a published work of Tôdô Ohara, entitled "Tôdô Ihitsu" (Remaining works of T.d.) Vol. 2 pt. 2 (Book 6) fol. 15 (published in Kaei 3, 1849), the Nankai Hofu was written by Yoshiyuki Murase, in the 11th month (lunar calendar) of Bunsei 1, or 1818. His material was based upon collections made by Ohara's disciples from various places in the province of Kii, now called Wakayama-ken. The work has never been published, but was revised and enlarged by Shinko Yamanaka, having a preface dated 1865. Illustrations (colored paintings) originally made by Kunihiko Uetsuji in 1818, were added to this revised manuscript, and the atlas has an independent title "Kankit-u Dzue" (Illustrations of citrus fruits) and bears the preface and postface dated 1864 and 1867 respectively. This revised work entitled "Zôtei Nankai Hôfu," with Uetsuji's atlas, is the only one known at present and exists only in manuscript copies. Fortunately the original preface of Y. Murase (left out entirely in the revised copy), the table of contents and a page of text of the original work with a figure painted by Uetsuji, were published in Ohara's work. This shows that Murase's original description of citrus varieties is in Chinese, while the revised copy of Yamanaka is all translated into Japanese except the quotations from Chinese books.

³ The measurement of height is omitted in the revised copy of Yamanaka.

(*Fei lo* in Chinese which means scrofula-ulcer)¹ on the rind looking very rough like a lion-head.² Even in mid-winter the peel remains green while the inside is quite red-ripe. It has a flavor similar to Kunembo, or better still, and the rind is as edible as in the latter variety . . . " It is also stated that this orange is locally called "Tô-kunembo" in the place where it is grown, but this name is often used, according to the author, to designate some other forms. So far as the illustrations of Uetsuji and the text of Murase go, this seems to be not a distinct variety of orange but merely a scabby Kunembo, such as are very commonly met with in the same locality at the present time.

Under the heading "Reishikitsu" (Lychee orange), the text of the revised manuscript Nankai Hôfu reads, "it resembles the Satsuma, but the peel is warty and has pimples (*Kotsutô* in Japanese, *ko ta* in Chinese)³ on it, looking like the skin of the lychee fruit. It tastes good . . . " The Japanese name given in the text is "Kasa Kôji" (Scabby Kôji orange), but the appearance of the fruit shown in the figure is quite far from typical thin-skinned yellow Kôji orange of very small size, illustrated elsewhere in the same work. The orange is very likely a scabby Satsuma. There is nothing else to which it can be referred.

These two kinds of scabby oranges were again treated in the well known work of Tsunemase Iwasaki, entitled "Honzô Dzufu." The work was completed in 1828 but it remained unpublished except for a few early volumes. The present facsimile edition⁴ presents good proof that the scabby oranges are really diseased fruits. Under "Reishikitsu," it states that the orange is in size that of Kôji and is round, having thick, stiff skin with yellowish white pimples on the surface, giving the appearance of the lychee skin. The appearance of fruits and leaves illustrated is entirely different from that of Nankai Hôfu, and gives an impression of figuring some round-fruited mandarin infected by Citrus scab. Under "Shitôkan," two fruits with very pronounced lesions of citrus scab are illustrated; the text says that the orange is commonly planted in Suruga province; the fruit resembles Yuzu somewhat flattened with a reddish-yellow skin covered with green pimples looking like the back of a toad; the pulp is sweet. A leaf

¹ see Giles Chinese-English dictionary, 2d ed., nos. 3468 and 7287.

² The lion-head, commonly impressed from the image carved in stone and placed in front of shrines, is very warty in appearance, as the name is represented by repeated tubercle protrusions covering the whole surface.

³ see Giles Chinese-English dictionary, 2d ed., no. 6050.

⁴ Iwasaki, Tsunemasa. Honzô Dzufu (Illustrated herbal). new ed. supervised by M. Shirai & K. Onuma. published by Honzô Dzufu Kwankôkwai, Tôkyô. see Book 62 fol. 7 & 11, issued T. 8, iv, Apr. 1919.

illustrated bears a broad petiole wing, but the text denies the presence of it, so that it is difficult to determine whether this is a Satsuma or a Kunembo.

There seems to be no scientific account in Japanese literature of the fungus causing the citrus scab before Nishida,¹ who gives *Cladosporium citri* Mass. as the causal organism. Shirai,² on the other hand, considers *Cl. elegans* Penz. to be the fungus causing "Mikan no Kasa" (Syphilis-scab of oranges). Yoshino³ apparently collected the specimens of both citrus scab and citrus canker. He gave *Cl. citri* Mass.? as the cause of scab, and *Cl. elegans* Penz.? as the cause of canker. Shirai and Miyake,⁴ however, mention both species as causing citrus scab, though many Japanese writers maintain that the real organism causing citrus scab is uncertain.

Citrus scab is distributed all over Japan, but the severity of infection is not equal in all places. According to the writer's field observations, the citrus groves in Ehime prefecture show almost no severe infections of citrus scab, while the orchards of Oita prefecture in Kyûshû, notwithstanding they are located only 30 miles away across the channel, are very severely infected.

The disease is also widely distributed in Formosa, as evidenced by the report of Formosan Agricultural Experiment Station,⁵ stating that Kankitsu Sôkabyô (*Cladosporium citri* Mass.?) affects leaves and young stems of citrus, collected from 20 different places in the island. Hori⁶ also mentions that the disease is common in Bonin and Formosa.

¹ Nishida, T. see work mentioned in note (5), p. 3.

² Shirai, Mitsutarô. Nippon Kinrui Mokuroku. A list of Japanese fungi hitherto known. Tôkyô, M. 38, xi, Nov., 1905. p. 21.

³ Yoshino, Kiichi. Higo Kokusan Kinrui (Fungi collected in Higo province) in Shokubutsugaku Zasshi, Bot. Mag. (Tôkyô) 19 no. 224: 219. M. 38, ix, Sept., 1905. (Japanese.)

⁴ Shirai, M. & Miyake, I. Nippon Kinrui Mokuroku. A list of Japanese fungi hitherto known. 2 ed. Tôkyô, T. 6, xi, Nov., 1917. p. 135-136.

⁵ Taiwan Sôtokufu Nôji Shukenjô (Governm. Gen. Form., Agr. Exp. Sta.) Taiwan Nôsakumotsu Byôgai Mokuroku. List of fungi on cultivated plants of Formosa, Part 1. (Division of plant pathology Bull. no. 1.) Taihoku, M. 41, ix, Sept., 1908. p. 39-40.

⁶ Hori, S. see work mentioned in note (4), p. 281.

TAXONOMY OF THE SCLEROTINIA ON HELIANTHUS ANNUUS L.

EDITH SLYMOUR JONES

WITH ONE FIGURE IN THE TEXT

OBSERVATION AND DESCRIPTION OF THE DISEASE

A wilting of sunflower plants was recorded from Wisconsin for the first time in July, 1918, when it was found at Madison. In 1919 and 1920 several groups of wilted plants were found at Ashland in fields where sunflowers were grown for silage.¹ An examination of the plants during the early stages of attack showed that the first evidence of the disease was a scalded area at the base of the stem which in moist weather was covered by a white mycelial mat. In dry weather, a wilting and burning of the leaves developed, followed a few days later by a rapid extension upward of the diseased area for a distance of about one foot. When the infected plant had completely wilted, sclerotia began to form on the outer surface of the stalk. The mycelium was found to have penetrated through the epidermis into the parenchyma surrounding the bundles and into the central pith where, as also among the roots, sclerotia had developed abundantly. In the diseased region the fungus had reduced the stem to a mere mass of vascular bundles surrounded by some disintegrated parenchyma. The disease was more prevalent where there was a thick stand of plants and where sunflowers had been grown successively for a number of years. The progress of the disease was favored by moist, cool weather. The soil in the Madison garden where the disease occurred was a clayey loam, quite moist, and very heavily fertilized.

OBSERVATIONS OF A SCLEROTIAL DISEASE ON SUNFLOWER IN AMERICA

American literature shows that a sclerotial disease of sunflowers has gained importance with increased sunflower culture in the northwestern states. As early as 1890 it was observed by Pammel (15) in Iowa. The plant disease surveys of the United States (8) and Canada (16) in 1921 both reported the common occurrence of the same disease, recording it in Oregon, Washington, Minnesota, and Ontario. The plant disease survey for Canada (10), published in 1922 contains a note by Cunningham who found *Sclerotinia libertiana* Fekl. on sunflowers

¹ These observations were made by Dr. J. G. Dickson.

and also records a root rot on sunflower seemingly distinct from the disease caused by *Sclerotinia*. Lawrence (13) in Washington, Morris and Swingle (14) in Montana, and Bisby (3, 4) in Manitoba have studied the disease. The description which they have recorded agrees with that of the disease observed by Dr. Dickson.

In regard to the taxonomy of the causal fungus, Pammel suggests that the sclerotia resemble those of *Sclerotinia sclerotiorum* (a synonym for *Sclerotinia libertiana* Fckl.). Morris and Swingle and Bisby agree that the causal organism resembles *Sclerotinia libertiana* Fckl. Bisby and Buller (5), in their list of Manitoba fungi, record what is considered to be *Sclerotinia libertiana* on *Helianthus annuus* (cult.). Lawrence, on the other hand, without referring to any previous observations of *Sclerotinia* on sunflower, describes the fungus as a new species under the name, *Sclerotinia perplexa*. His description differs from that of *S. libertiana* as given by Fuckel only in the addition of a conidial stage which he found upon the host plant and from which he grew sclerotia in culture. Since, except for Lawrence, the Americans have not clearly stated the taxonomy of the fungus causing the disease, it seemed worth while to study the fungus with a view to determining its taxonomic position.

INOCULATION EXPERIMENTS AND DEVELOPMENT OF THE PERFECT STAGE

The fungus isolated from the diseased sunflower stalks was inoculated into young sunflower and lettuce plants. In the sunflower the disease which was observed in the field was readily reproduced (FIG. 1). The lettuce wilted rapidly, showing the typical symptoms of lettuce drop. Sclerotia obtained both from sunflowers in the field and from inoculated plants were placed for overwintering below the surface of moistened quartz sand in covered flower pots which were buried in the garden two feet below the surface of the soil. In May the sclerotia were found germinated.

The following is a description of the perfect stage made unfortunately after the apothecia had been kept for some time in alcohol.

Fructing bodies numerous, stipitate, smooth, light-brown, funnel-shaped, 0.9 to 3.0 μ in diameter; stipe slender, straight, sometimes bent one or more times; sclerotia irregular tending in general to be somewhat tuber-like in form, at times elongate, black externally although sometimes covered with white mycelium, cream colored within; asci cylindrical, hyaline, 66 to 136 by 6 to 7.4 μ ; spores monostichous, ellipsoid 7.4 to 11 by 3.7 to 4.6 μ ; paraphyses filiform.

This description agrees closely with Fuckel's description of *Sclerotinia libertiana* as quoted by Saccardo (17). The most important difference

between this description and that of Fuckel consists in slightly smaller spore measurements, the spores under observation measuring 7.4 to 11 by 3.7 to 4.6 μ in contrast with Fuckel's measurements 9 to 13 by 4 to 6.5 μ . In this connection however it is of interest to note that



FIG. 1. The effect of inoculation of sunflower plants with *Sclerotinia libertiana*. Photograph taken 12 weeks after inoculation. Uninoculated plant at left; a sclero-

deBary (2) found that fresh spores of *S. libertiana* measured 11 to 12 by 4.5 to 6 μ whereas spores from material dried or kept in alcohol were only about 9 by 4.5 μ . DeBary's measurements of ascospores preserved in alcohol are identical with the mean of the measurements given in the description above, thus leaving no reason to doubt that this fungus is *S. libertiana*.

In connection with this determination of *Sclerotinia libertiana* on sunflower it is of interest to note that there are many European records of this fungus upon the same host. As early as 1861 Fuckel (9) describes the perfect stage of *Sclerotium compactum* DC. b. *Helianthi* Rabh. *Sclerotium compactum* DC. was later shown to be the sterile condition of *Sclerotinia libertiana* Fuckel. In 1876, Brefeld (6) describes the parasitizing of *Helianthus tuberosus* by a *Peziza*-forming sclerotium. Frank (7) in 1897 records *Sclerotinia libertiana* on the same host. DeBary (2) in 1886 found the same fungus twice on stalks of *Helianthus annuus* although he says that *Sclerotinia* rarely occurs on *Helianthi*. Kirchner (12) records *S. libertiana* on sunflower in 1897. Saccardo (17) gives *S. libertiana* on *Helianthus annuus*. Of the two more recent observations, Jachevski (11) in 1921 in Russia records the continual extension of injury to sunflower and other kitchen-garden plants from *S. libertiana*, and the Journal of the Board of Agriculture (1) of Great Britain in 1917 publishes a record of sunflower as a host of *Sclerotinia sclerotiorum* (syn. *S. libertiana*). The evidence brought together here indicates beyond a doubt that the disease of sunflower of Europe caused by *Sclerotinia libertiana* is identical with that which has been encountered most frequently in America.

MADISON, WISCONSIN.

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FUNGI ISOLATED FROM THE INTERIOR OF COTTON SEED

R. F. CRAWFORD

The cotton seed used in this experiment was selected from badly wilted plants during the later part of September, 1921, by Dr. J. A. Elliott of the Arkansas Experiment Station. The chief object of the experiment, as reported by Elliott (3), was to determine whether or not cotton wilt was carried in the seed. Several other fungi made their appearance from time to time during the experiment, some of which have not been reported as being seed borne. The cotton seed was delinted with concentrated sulphuric acid, washed thoroughly, dried and stored in a cloth bag. At the time of planting the seed was again charred in sulphuric acid, treated for two minutes in a 1-1000 solution of mercuric chloride, and washed in sterile water. The plating was done on white blotting paper, which was dampened, placed in petri dishes, and sterilized in an autoclave for one hour at 15 pounds pressure. It would seem hardly possible that any external organisms could survive the treatment given the cotton seed, and all fungi reported are considered as coming from the interior of the seed.

TABLE 1—*Fungi isolated from cotton seed.*

Series	Date	No. of seeds	<i>Fusarium vas-infectum</i>	<i>Colletotrichum</i>	<i>Diplodia</i>	<i>Fusarium</i> No. 1 ¹	<i>Fusarium</i> No. 2 ¹	<i>Fusarium</i> No. 3 ¹	<i>Cephalothecium</i>	<i>Alternaria</i>
A	9/27/21	105	4	11	1	0	4	0	1	0
B	2/28/21	119	5	13	1	0	7	1	1	0
C	10/ 6/21	150	6	14	0	0	5	1	0	0
D	10/16/21	150	5	11	0	2	1	0	0	2
E	10/25/21	150	5	18	0	8	1	0	0	0
F	12/12/21	95	3	20	0	17	0	0	0	0
G	1/17/22	100	1	20	0	23	6	0	0	0
H	2/16/22	200	4	72	0	30	7	0	2	0
I	3/14/22	200	1	54	2	28	3	0	2	0
J	4/17/22	200	1	41	9	15	14	0	1	1
Total		1469	46	274	13	123	48	2	7	3

¹ With the exception of *Fusarium vasinfectum*, no specific name has been given to the *Fusaria* found. Cultures of each have been sent to Dr. C. D. Sherbakoff for identification.

The increase in the number of isolations of certain of the fungi in the later platings was undoubtedly due to the fact that at first only apparently viable seed was selected, while in the last five platings the seed was unselected and contained a considerable amount of dead and shriveled seed which was naturally more apt to carry the facultative parasites or saprophytes.

Colletotrichum.—To establish the pathogenicity of the *Colletotrichum* sp. isolated from the interior of the cotton seed, healthy cotton bolls in various stages of maturity were brought into the laboratory and inoculated with a pure culture of the fungus. These inoculations were made by transferring some of the spores into a wound in the boll made with a sterile knife. Successful inoculations producing the characteristic anthracnose boll-rot were obtained in all cases, as had been done in experiments by Barre (1), Edgerton (2), and others with *Glomerella gossypii* isolated from the interior of the cotton seed.

Diplodia gossypii.—The disease caused by this fungus was discovered and described by Edgerton (2) of the Louisiana Experiment Station, but no mention is made in the literature of its being seed borne. Elliott (4) states the possibility of its being carried on the seed. All the cultures of *Diplodia* were isolated from the interior of dead seed. A number of cotton bolls were inoculated by means of needle punctures with pure cultures of the fungus. In a few days after inoculation, the bolls first became brown, and shortly after, pycnidia and spores could be found on the surface. The entire boll would soon be covered with a jet-black powdery coating of spores. The rot progresses very rapidly, involving the entire boll and destroying the lint. Dr. C. W. Edgerton, in conversation with Dr. J. A. Elliott, suggested the possibility that cotton and sweet potato *Diplodia* were similar, if not identical. Inoculations were made on sweet potatoes with the cotton *Diplodia* and positive results were secured. The disease did not progress nearly as rapidly on the sweet potatoes as on the cotton. The sweet potatoes were slowly rendered dry, hard, and brittle, with a coal black interior. The typical pycnidia and two-celled brown spores were found on the surface of the sweet potato.

Fusarium No. 1.—This *Fusarium* has a peculiar lemon-shaped sporangium-like body and a very coarse mycelium. It has been under observation for some time and produces macroconidia very sparingly. On rice the *Fusarium* produces a dark brown color. This fungus is evidently the same as has been observed by Dr. C. W. Edgerton of the Louisiana Experiment Station. In a letter Dr. Edgerton states: "I remember distinctly of obtaining a fungus with peculiar sporangium like bodies,

which I was never able to develop any further." No inoculation experiments have been conducted with this *Fusarium*.

Fusarium No. 2.—This fungus, commonly found on and in cotton seed, appeared to be identical with the one reported by Edgerton (2) as very common on rotting bolls, especially in wet weather. The mycelium is apparently able to grow into the kernel of the seed and remain in a dormant condition during the winter. In the spring when the seed is planted the fungus forms spots on the cotyledons. This *Fusarium* was isolated from spots on the cotton leaves both in the field and greenhouse as well as from the interior of cotton seed. Field observations would indicate that a large percentage of young seedlings have these *Fusarium* spots on the cotyledons. Inoculated bolls show a slight staining of the lint and eventually may become entirely rotted. Injury to the bolls, such as caused by insects or the angular leaf-spot organism, is apparently necessary for infection.

Fusarium No. 3.—The first mention in literature of this *Fusarium* is by Elliott (4) who describes it as causing a rapidly progressing bright pink rot of cotton bolls. Throughout the experiment, some of the young seedlings, that did not show any fungous growth, were removed from the petri dishes and transferred to test tubes containing an inch of agar. Two of these plants developed fungous growth which stained the potato agar slants, on which they were growing, a bright pink far below the growth of the mycelium. Cotton bolls in all stages of maturity were successfully inoculated in the laboratory. The rot progressed with great rapidity and soon destroyed the whole boll, producing a characteristic bright pink color of the lint. This *Fusarium* produces a rot of the bolls about as quickly as does the *Diplodia* boll-rot fungus.

Two other fungi, a species of *Cephalothecium*, and an *Alternaria*, were isolated but no studies or inoculation experiments were conducted with them.

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PHYTOPATHOLOGICAL NOTES

Gum diseases of sugar cane in the Philippines. Miss Wilbrink¹ has pointed out the occurrence in Java of a bacterial disease, the symptoms of which are red vascular bundles, sudden wilting of the leaves from the tips in longitudinal whitish streaks, and the production of numerous shoots from the lateral buds of the standing cane. The disease is identical to Cobb's gum disease of sugar in Australia with the exception that in Java there is no yellow bacterial ooze from the cut vascular bundles as is the case in Australia.

In a previous publication, Lee² reported an unidentified disease from the Philippines which for convenience was at the time termed red-vascular disease. The identity of the disease was uncertain at the time, due to the absence of the yellow ooze reported for gum disease by Cobb³ and others.

A recent visit to Java has shown the identity of the Philippine red-vascular disease with the bacterial cane wilt in Java; there is the same whitish longitudinal streaking of the leaves, sudden wilting, reddened vascular bundles and varietal susceptibility. Bacteria can be seen in masses in sections of the vascular bundles of affected cane under the microscope. Bacteria show up uniformly in dilution plates which agree with Miss Wilbrink's description of the Java vascular organism, although our inoculation experiments are not sufficiently extended in time to enable us to state that we have reproduced the disease by inoculation. This disease in our experience in the Philippines attacks the varieties H-109, Lahaina, Negros Purple and Louisiana Striped very severely. We have not seen it on the Badila variety nor on the Caledonia canes.

With the determination of this disease we are able to point, not entirely with pride, to the presence in the Philippines of all but two of the important diseases of sugar cane known in the literature. These two, sereh and Lahaina disease, may show up with further experience.—H. ATHERTON LEE.

¹ Wilbrink, Miss G. De gomziekte van het suikerriet, hare oorzaak en hare bestrijding. Archief voor de Suikerindustrie in Nederlandsch-Indie 28: 1399. 1920.

² Lee, H. Atherton. Observations on previously unreported or noteworthy plant diseases in the Philippines. Phil. Agr. Rev. 14: 432. 1921.

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Leaf cast of Larix occidentalis by Hypodermella laricis Tubeuf in north Idaho. The disease of western larch known as leaf cast (*Hypodermella laricis* Tubeuf) has been previously reported from Idaho by various writers. However, it appears as if the importance of the disease has not



FIG. 1 *Hypodermella laricis* killing short spurs of *Larix occidentalis*.

received sufficient attention. The purpose of the present note is to emphasize the fact that *Hypodermella laricis* does more damage than merely killing the needles in that it very often kills great numbers of the short spur-like branches which bear the needles.

The common name "leaf cast of larch" as applied to this disease is a misnomer in that exactly the opposite phenomenon is produced. The

infected leaves instead of being deciduous remain on the tree until the following year and become quite conspicuous early in the growing season in contrast with the new leaves.

During the last four years, the writer has noted the general distribution of the disease in north Idaho but during the summer of 1922 the infection appeared to be particularly severe. An idea of the severity of the infection may be conveyed by the appearance of a young stand of larch in the small sapling stage in midsummer. Last year many such stands appeared as if a fire had passed through them and these stands, when viewed from a distance, did not even have a greenish cast.

On examining such a stand again this spring after the new growth of needles had been produced, it was observed that many of the short spur-like branches, which still had last year's infected needles attached to them, were not producing new needles and that they had been killed. A count of five branches, picked at random, bore a total of 593 leaf-bearing spurs of which 29 per cent or 172 had been killed. On one of these branches having a total of 199 leaf-bearing spurs, only 121 were producing needles, the remainder, 78, had been killed. Dead branches are also quite common on badly infected trees and there is little doubt that they had been killed by the fungus under discussion.

This reduction in the leaf area must be reflected in the rate of growth of the infected trees. In the writer's opinion *Hypodermella laricis* causes an important disease of western larch and should be given detailed study. In figure 1 the dead, leaf-bearing spurs with last year's infected needles are plainly visible. This specimen was collected May 11, 1923.—HENRY SCHMITZ.

THE FIFTH ANNUAL SUMMER MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The fifth annual summer meeting of this Society, which was held in western New York and Ontario, Canada, during the four days from July 9 to 12, 1923, inclusive, was most successful. The weather was perfect; the attendance was good, there being present sixty-nine members of the Society and about twenty guests; the automobile transportation facilities were excellent; and the program, arranged by the Advisory Board, with M. F. Barrus and J. H. Faull in direct charge of the itineraries for New York and Canada, was interesting and well conducted. The two local committees who had charge of the plans deserve much credit and have the thanks of the Society for their commendable work.

On the first day of the meeting the gardens, orchards and equipment of the New York Agricultural Experiment Station at Geneva, and experimental apple orchards in the vicinity were inspected. On the following day automobiles conveyed the party from Geneva to Rochester by the way of Lyons, Sodus, Williamson, and Irondequoit, frequent opportunities being provided for observing diseases of wheat, raspberries, apples, cherries, lettuce, onions, greenhouse cucumbers, tomatoes, and other crops. On Wednesday, July 11, the tour was continued through the extensive western New York fruit belt to Niagara Falls by way of Hilton, Albion, Medina and Middleport. During this part of the trip apples, cherries and raspberries particularly were examined for diseases. On July 12 the party was entertained by officials of the Canadian Department of Agriculture, the Ontario Agricultural College and the fruit growers of the Niagara Peninsular section. Stops were made at about seven places between Niagara Falls and Hamilton, Ontario, including a visit to the Experiment Station of the Province of Ontario at Vineland. The large variety of fruits that are grown very intensively in this section, although in general remarkably free from diseases, were of much interest to the visiting pathologists. The meeting culminated with a complimentary banquet provided by the Dominion Department of Agriculture at the Royal Connaught Hotel in Hamilton.

One of the main features of the meeting was the excellent opportunity provided for studying raspberry diseases of the yellows type, that is, mosaic, leaf curl, and the so-called eastern blue-stem or rosette. Plenty of diseased plants were available for study and the pathologists who have done the most work on these diseases were present to give explanations concerning them. A field where roguing for control of mosaic and leaf curl has been practiced was remarkably free from the disease.

Another important line of work, the results of which were inspected in four orchards, was the apple spraying and dusting experiments of the Geneva and Cornell Experiment Stations. Among the preparations under investigation were sprays and dusts to which various kinds of stickers had been added, colloidal and precipitated sulfur as prepared by H. C. Young, and nicotine sulfate and tobacco dust in combination with various sprays and dusts for red bug control. In the Cornell experiment at Sodus a very good example of the importance of the timeliness of the pre-blossom spray for scab control was noted.

At Williamson, N. Y., the onion breeding and smut control plots were of much interest, and the visits to the Niagara Sprayer Company at Middleport, N. Y., and the fruit packing plant of Senator E. D. Smith at Winona, Ontario, deserve especial mention.

Of the diseases that were observed, perhaps the following were most interesting: mosaic, leaf curl, eastern blue-stem, powdery mildew (undet.) and cane blight (*Lep-tosphaeria coniothyrium*=*Coniothyrium fuckelii*) of raspberry; orange rust (*Kunkelia nitens*) of blackberry; *Fomes rubis* on currant; leaf spot (*Mycosphaerella fragariae*), leaf scorch (*Mollisia earhana*), root rot (undet.) and winter injury of strawberry; fire blight and scab on apple; leaf spot (*Coccomyces hiemalis*) of cherry; mosaic, tipburn, bottom rot (*Rhizoctonia* sp.), drop (*Sclerotinia libertiana*) and Botrytis rot (*Botrytis* sp.) of lettuce; smut (*Urocystis cepulae*) and pink root (*Fusarium* sp.) of onion; and take-all and other diseases of wheat. The stem nematode (*Tylenchus dipsaci*) was found for the first time in the eastern United States on the common dandelion by G. H. Godfrey during the course of this field trip.

The following resolution, presented by a Resolutions Committee, was adopted:

To whom it may concern:

The members of the American Phytopathological Society appreciate very much the many courtesies shown them by the different groups and individuals during the occasion of the Fifth Annual Summer Meeting of this Society, and we hereby formally wish to express and convey that appreciation, especially to the officers and staff of the New York Experiment Station, Geneva, to the staff of the Extension Service and Plant Pathology Department of the Agricultural College, Cornell University, under the kindly and efficient leadership of Profs. M. F. Barrus and H. E. Thomas, to the Dominion of Canada Department of Agriculture and to the Ontario Department of Agriculture, especially the officers and staff of the Vineland Experiment Station, to the Agricultural Representatives and fruit growers of the Niagara District, Ontario, to Prof. J. E. Howitt, Ontario Agricultural College, Guelph, to the Monroe County Farm Bureau under the leadership of Mr. E. G. Smith, to the Sodus Fruit Farm, Inc., Sodus, N. Y., to the Hon. Senator E. D. Smith, Winona, Ontario, to Mr. F. L. Strickland, Lockport, N. Y., and to the Niagara Sprayer Company.

The Committee on Resolutions requests that this note of appreciation be spread on the minutes of the Society and a copy be sent to each of the above-mentioned organizations and individuals.

Signed:

S. M. ZELLER, Chairman.

CHARLES CHUPP,

G. H. COONS,

Resolutions Committee.

R. J. HASKELL,

SECRETARY, ADVISORY BOARD

OF AMERICAN PLANT PATHOLOGISTS.

ABSTRACTS OF PAPERS PRESENTED AT THE FIRST ANNUAL MEETING OF THE INLAND EMPIRE SECTION, PACIFIC DIVISION, AMERICAN PHYTOPATHOLOGICAL SOCIETY, PULLMAN, WASH., AND MOSCOW, IDAHO, FEBRUARY 3 AND 10, 1923.

At a meeting held at Pullman, Washington, February 3, 1923, at which were present the plant pathologists of Washington State College and the University of Idaho, the following program was given by the members from Washington State College.

Botrytis diseases in Washington. B. F. DANA.

The following diseases as they have occurred in Washington have been assigned to *Botrytis cinerea* Pers: Apple fruit rot, carrot storage rot, cranberry fruit rot, fig canker, ginseng moldy seed, gooseberry dieback, lettuce gray mold, lilac leaf blight, peony blight, pear, plum, raspberry, and strawberry fruit rots, and squash storage rot. Onion neck rot, *B. alii* Munn., peony blight, *B. paeoniae* Oud., and tulip blight, *B. parasitica* Cavara have also occurred in Washington within the last eight years. During the same time the following diseases caused by unassigned species of *Botrytis* have occurred: eggplant fruit rot, golden seal leaf blight, periwinkle leaf blight, rose blossom blight, and snowball blossom blight.

Notes on Rhizoctonia. B. F. DANA.

Experimental work on potatoes at Pullman has shown that selection of visibly clean seed stock will give the largest quantity of table stock. If this clean seed is treated with mercuric chloride it will increase the yield of clean tubers in the table stock. In one experiment infected untreated seed gave 9 per cent, infected treated, 59.5 per cent, and clean treated seed gave a yield of 80 per cent clean table stock. Mercuric chloride has proven superior to formaldehyde against *Rhizoctonia*. The following results of one test are representative. Infected untreated seed yielded 50 per cent clean table stock. Treatment with formaldehyde and mercuric chloride increased the yield of clean table stock to 73 and 84 per cent respectively. Treatment of seed with mercuric chloride, four ounces to thirty gallons of water for two hours, has not increased yields in potatoes treated just before planting. Shortening the time of treatment gave lessened control even when double strength or eight ounces of mercuric chloride to thirty gallons of water was used.

Spraying with Bordeaux mixture at Pullman and Yakima and soil fertilizer treatments at Pullman did not consistently increase the yield or per cent of clean tubers in the crop. Late spring plantings at Pullman gave a higher yield of clean tubers in the crop from infected seed than early spring planting under the same conditions.

Preliminary report on rots of the cranberry in Pacific County. D. J. CROWLEY.

During the past season the writer made an investigation of the fungous diseases of the cranberry in Pacific County, Washington, and also carried out a series of spraying experiments to determine the best means for their control. Very little rot showed in the bogs during the growing season, but soon after the berries were placed in storage,

they started breaking down rapidly. Later work in Pullman showed that the following organisms were responsible for the storage rots: *Fusicoccum putrefaciens* Shear; *Gurgnardia vaccinii* Shear, *Pestalozzia guenivii vaccinii* Shear, *Sporonema oxycocci* Shear; *Sporonema pulvinatum* Shear; *Anthostomella destruens* Shear; *Phomopsis* sp.; *Stemphylium* sp.; *Botrytis* sp. Storage of berries at 32° F. reduced the storage losses by about one-half. Further investigations are still being carried on.

Coryneum blight of stone fruits in Washington. CHAS. S. PARKER.

This disease was shown to be of economic importance periodically in parts of the State of Washington. By means of artificial inoculations and re-isolations all of the typical symptoms of the disease as observed in the orchards were reproduced within the greenhouse or laboratory. Experiments involved the use of peach, apricot and cherry hosts. *Coryneum beijerinckii* Oudem. was demonstrated to be the causal organism. Infection was found to take place only through the stomata or ruptured epidermal tissue of leaves. The fruiting body was shown to be an acervulus, which was readily reproduced on twigs of the host plants, but never on dextrose or prune agar. Spores upon the natural matrix will resist unfavorable conditions for a very extended period. After nineteen days of desiccation spores germinated within twenty-four hours.

Notes on the anthracnose of lettuce. CHAS. S. PARKER.

In the fall of 1922 volunteer head-lettuce plants showing this disease were transferred in flats from the open garden to greenhouse. *Marssonina panattoniana* (Berlese) Magnus was isolated and grown on 3 per cent dextrose agar plus ten. During the winter months, by means of an atomizer, spores from the pure culture were sprayed over the leaf surfaces of *Lactuca scariola* L., which were growing in the greenhouse, and a perfect infection was obtained. Early in the spring of 1923, typical anthracnose lesions were observed along the mid-rib of *Lactuca scariola* L. growing in the open as a weed. Isolations made from this material were growing well in pure culture when a sudden increase in temperature due to exceptional warm weather killed this material and the pure cultures from the cultivated lettuce.

Fusarium rot of onions. L. W. BOYLE.

A brief statement was presented of the onion rot problem of the Walla Walls truck district of Washington. The rot occurs in the field but is most important in storage and transit, causing heavy losses. Specimens from the field showed wilted and brown soft tops. The bulbs, however, showed no rotting about the necks except in very advanced stages. Certain specimens appeared perfectly normal until cut. Starting at the base and extending various distances up individual scales, the tissues had a solid water-soaked appearance. The soft rots were likewise limited to individual scales and the tissue had a brown slimy appearance. This condition also started at the base and was apparently following up the individual scales after the before-mentioned water-soaked condition. The roots were still attached but in many cases easily brushed off. Specimens of the rot in the advanced stage, where a large part of the bulb was soft, had a heavy ring of white fungous growth about the base. This invariably was producing *Fusarium* spores. Bacteria and fungi were isolated from the soft brown rotted tissue near the base of bulb scales, and pure *Fusaria* cultures were isolated from the more solid tissue at the edges, in advance of the soft brown tissue. Methods of isolation and inoculation were described. Further work is necessary to determine whether bacteria or fungi alone, or together, cause the rot.

Further observations on the relation of the spore load to the per cent of bunt appearing in the crop. F. D. HEALD AND L. W. BOYLLIE.

To be published in full in PHYTOPATHOLOGY.

The copper carbonate bunt problem. F. D. HEALD.

A brief review was presented of the work carried out on the copper carbonate dust treatment for bunt or stinking smut of wheat, with a presentation of the features in which the new treatment shows a superiority over the old wet methods of seed disinfection with bluestone or formaldehyde. Special emphasis was given to the complete elimination of seed injury by the use of copper carbonate. Several lots of seed treated a year ago gave better germination than untreated seed. Copper carbonate treated grain invariably has shown a greater vigor of growth and the protection against smut has been better than when formaldehyde is used, and equal to or better than when bluestone has been employed.

Many different commercial brands of copper carbonate are now on the market and one of the present problems is the determination of what specification should be demanded. The different brands differ in purity, specific gravity and fineness. Two ounces of Corona Compound per bushel, testing from 17 to 21 per cent of metallic copper, has given practically as good protection in spring plantings as pure copper carbonate, testing fifty or more per cent of metallic copper.

The following week, February 10, 1923, those interested in plant pathology, from the two institutions, convened at Moscow. At this meeting the final organization of the Inland Empire Section, Pacific Division, of the American Phytopathological Society was completed. There were twenty-six present. The following officers were elected:

President, F. D. Heald.

Vice-President, C. W. Hungerford.

Secretary-Treasurer, J. M. Raeder.

The following program was given by the Idaho representatives:

Studies in wood decay V. Physiological specialization in Fomes pinicola Fr. HENRY SCHMITZ.

The results obtained from a physiological study of four cultures of *Fomes pinicola* Fr., obtained from four different hosts, namely, Douglas fir, (*Pseudotsuga taxifolia*), white fir (*Abies grandis*), western hemlock (*Tsuga heterophylla*), and western white pine (*Pinus monticola*), were reported. The results obtained show that these four strains of *Fomes pinicola* differ very markedly in (1) the characteristics of growth, (2) the rate of growth, (3) the extracellular enzyme activity, (4) the intracellular enzyme activity, (5) the effects produced in mixed cultures, (6) the growth in liquid media and (7) the nitrogen relations.

The distribution of Ribes and five-needle pine in the Okanogan region of Washington and British Columbia. V. H. YOUNG.

Preliminary results of experiments with leaf roll and mosaic in Idaho. CHAS. W. HUNGERFORD.

Bliss Triumph potatoes from mosaic free stock were grown under cloth cages and various insects were introduced into these cages after having fed upon mosaic diseased plants. The following insects were used: green peach aphid (*Myzus persicae* Sulz),

pink and green rose aphid (*Macrosiphum solanifolii* Ashmead), a false chinch bug (*Nysius ericae* Shilling), tarnish plant bug (*Lygus pratensis* Linn.) and several others. Positive results were secured with the pink and green rose aphid only. Characteristic mottling developed three weeks after the aphids were introduced into the cages. All the checks remained healthy. Tubers from these infected plants were later grown in the greenhouse and in each case at least some of the tubers from each hill produced badly diseased plants. In one case four small tubers from one of these plants were planted in the greenhouse. Two of these produced apparently healthy plants while the other two were badly diseased. Small potato seed plots grown in various parts of the state from selected seed of the same seed lot developed varying amounts of mosaic when grown in different localities. This apparently would indicate either that mosaic symptoms were masked under some climatic conditions or else local sources of infection from other fields or from wild hosts were present in some cases and not in others. This work is being continued upon a larger scale this year.

Results of tests with copper carbonate in Idaho. CHAS. W. HUNGERFORD.

Tests have been made the last two years with several dust treatments for the control of stinking smut. None of these treatments have given as good control of smut as the standard bluestone treatment under the conditions where they have been tried. Three different commercial brands of copper carbonate were used. None of these gave as good control as bluestone. Germination, however, was better in every case where copper carbonate was used. In small experimental plots the stand was better in each case where copper carbonate was used than in the check plots. These tests are being continued in cooperation with a large number of farmers in various parts of the state using a number of commercial brands of copper carbonate and a number of mercury compounds.

The effect of pre-sprinkling with water on the hot formaldehyde and corrosive sublimate methods of potato seed treatment. J. M. RAEDER.

Further laboratory results this year indicate that the efficiency of both the hot formaldehyde and the corrosive sublimate treatments for seed potatoes is increased by first sprinkling the potatoes with water and then covering them for 24 or 48 hours. As compared with last year's results, this difference was not so marked. Results last year showed complete control with six different methods of treatment with hot formaldehyde subsequent to sprinkling. This year absolute control was obtained in only four such cases. The results of this work will soon be published in full.

Preliminary results with the use of sulfur for the control of potato scab in Idaho. J. M. RAEDER.

This work was conducted on but one acre of ground divided into thirteen plots, five of which were checks. The average pH value of the soil of these checks was 6.74. Although the pH value of the soil of some of the plots was materially reduced, the results obtained at digging time showed that control was negative. This work will be conducted on a larger scale during the coming year.

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NOTES ON THE FAILURE OF GRAPEVINES TO SET FRUIT AND ON SHELLING

ARTHUR S. RHODES

WITH PLATE XXI

The failure of grapevines to set fruit, or to retain their fruit after it has developed more or less fully is a trouble of considerable economic importance. Both this non-setting of grapes and their falling off after they have developed more or less fully is termed "coulure" by the French viticulturists.

While the failure of the flowers to set fruit, or of the berries to develop appreciably, has long been recognized by European viticulturists, and some phases of it by American horticulturists engaged in grape-breeding experiments, this trouble has been given but scant attention by American pathologists. From a consideration of the American pathological literature the impression is gained that the non-setting of grapes is of but little importance in comparison to the falling of the more or less fully developed fruit, or "shelling" or "rattles," as this trouble is commonly designated in this country.

Observations by the writer indicate that the non-setting of grapes is of far greater importance as a factor of loss than the commonly mentioned shelling. The undue prominence which shelling has attained in comparison to non-setting is largely due to the much greater conspicuousness of the loss caused by the former, the falling of some of the well-developed berries attracting the attention of even the most casual observer, whereas large numbers of the flower clusters may fail to set fruit or even blight and drop off entirely within a few days and entirely escape the casual observer or excite but little comment as a rule.

In California, Bioletti (1) has applied the term "early coulure" to the failure of the flowers to set fruit or the dropping of the berries without developing appreciably, and the term "late coulure" to the falling or shelling of the berries after they are well developed. Bioletti's distinctions seem very appropriate and should prove useful, especially in the absence of any other term, for the earlier form of this grape trouble.

The non-setting of the grapes, or early coulure, if we accept the more convenient term, has been investigated by a large number of observers and a variety of causes assigned. These may be either constitutional or accidental.

Under early coulure as a result of constitutional causes by far the most important factor to be considered is the lack of self-fertility of certain varieties due to imperfect development of the flowers or to lack of functional activity of the pollen produced. The work of a number of investigators has shown that a large number of grape varieties are more or less incapable of setting fruit if planted alone, but that, like the pistillate strawberries, they must depend upon the pollen of other varieties for perfect fertilization and fruitfulness. In other instances, flowers may be seemingly perfect in their organs and yet fail to develop fruit. Müller-Thurgau (6) regards this as an inheritable character of certain vines, the only remedy for which appears to be ringing the vines, which tends to lessen the amount of water supplied and to concentrate the liquid nourishment carried to the flowers.

Early coulure as a result of accidental causes has been attributed to such factors as unfavorable weather conditions or fungous attacks occurring at the flowering time, spraying while in bloom, improper pruning, a deficiency in the supply of reserve materials in the canes due to its depletion by excessive vegetative growth or by overbearing the preceding year, unsuitable or exhausted soil, and the absorption of excessive material from soils extremely poor in nitrogen and phosphoric acid. The writer wishes to record observations on the first two of these factors.

In 1922 all the grapevines in the vineyard of the Missouri State Fruit Experiment Station that had come into full bearing, produced a very satisfactory crop of fruit and in the spring of 1923 the same vines produced an abundance of blossom clusters and had every indication of bearing an equally good crop. However, the weather conditions that prevailed during the blooming period of the grapes resulted in a more or less severe reduction of the fruit upon certain varieties. This was occasioned largely by the failure of the fruit to set but was aided somewhat in a few varieties by the early development of black rot, which attacked the flower clusters even before the blossoms opened.

The observations made upon the station vineyard, at least those in so far as the counts were concerned, were confined to that portion of the vineyard in full bearing, which consisted of several different varieties in their fifth year. The Champion, which is the earliest bloom-

ing variety, was in full bloom on May 30, and the Carman, which is a very late blooming variety, had passed the climax of its bloom on June 18.

The fruit loss due to failure to set as a result of the prevalence of rain during the blooming period, estimated after the set of the remainder had been assured and checked up later in the season after the berries had become about half grown, was as follows:

Variety	Period of Blooming	Per cent Loss
Champion	Early	Less than 1
Cottage	"	2
Barry	Midseason	Less than 1
Concord	"	Less than 1
Daisy	"	4
Eclipse	"	25
Hartford	"	4
Herbert	"	5
Hernito	"	20
Hicks	"	2
King	"	4
Moore's Early	"	50
President	"	5
Captain	Late	20
Cloeta	"	1
Fern	"	50
Lomanto	"	5
Muench	"	80
Carman	Very Late	35

The unfertilized berries either fell off the flower clusters before attaining any appreciable development or else developed very tardily as compared to normal berries, and then fell off while still very small (Pl. XXI fig. 1). This same trouble was noted also in other small grape plantings at Mountain Grove and in vineyards in other sections of Missouri.

In four varieties the estimation of the fruit loss was complicated by the occurrence of blossom blight caused by the black-rot fungus, *Guignardia bidwellii* (Ell.) V. & R. The flower clusters attacked by this fungus quickly became brown, soft and rotten, the lower end being the first to become infected as a rule (Pl. XXI fig. 2). Within a few days the entire flower cluster became involved and dried up and dropped off, the scars left by the abscised peduncles being the only indication that the shoots had developed flower clusters. Numerous pustules of the black-rot fungus appeared in the bases of more or less of the new growth of the four varieties infected and, in a few cases, occurred even on the peduncles of the flower clusters. In addition to the figures already given for fruit

losses as a result of the prevalence of rain during the blooming period it was estimated that there was a further loss of 5 per cent of the potential crop of the Moore's Early, 50 per cent of that of the Hernito, 75 per cent of that of the Captain, and 35 per cent of that of the Carman due to black-rot alone.

An inspection also was made of three species of wild grapevines which occur abundantly on land adjoining the station. Of these species, which were in full bloom on June 20, blossom blight caused by black-rot was abundant on *Vitis aestivalis* Michx. and *V. cordifolia* Michx., practically all the flower clusters on some vines rotting back from the tip, and later turning brown, shrivelling and falling off. No evidence of black-rot was to be observed on any part of the vines of *V. cinerea* Engelm., even though growing in association with heavily infected vines of the other two species, this species appearing to be quite immune.

The meteorological records taken at the station, which are given below for the blooming period of the grapes, show that cool, rainy weather prevailed throughout the blooming period.

Date	Temperature		Precipitation in inches	General character of day
	Maximum	Minimum		
May 28	78	58	—	Partly cloudy
29	81	62	—	Clear
30	83	60	.09	Partly cloudy
31	85	62	trace	" "
June 1	86	65	trace	Clear
2	79	61	—	Partly cloudy
3	83	62	1.04	" "
4	79	64	.58	Cloudy
5	82	63	—	Partly cloudy
6	81	62	—	" "
7	83	61	.28	" "
8	83	60	—	" "
9	80	62	—	Clear
10	73	63	.67	Cloudy
11	75	64	.47	"
12	77	57	.18	"
13	69	53	trace	"
14	79	59	—	Clear
15	77	63	—	Partly cloudy
16	86	65	.08	" "
17	84	77	.46	" "
18	85	66	.03	" "
19	86	67	—	" "
20	84	67	—	Clear

Hedrick (3, p. 77) concludes that rain and the cold and wind that usually accompany it at blossoming time cause the loss of more fruit in New York than any other climatal agencies. The damage is done in several ways. In addition to the most obvious injury, namely, the washing of the pollen from the anthers, the secretion of the stigmas, which is so essential to the proper germination of the pollen, also is washed away or becomes so diluted that the pollen does not germinate. It is further probable that the chill of the rainy weather decreases the vitality of the pollen. Hedrick (4, p. 368) also states that further investigations at the Geneva Station have shown that the pollen grains of practically all fruits burst and will not germinate in water. From this work he is of the opinion that there can be little doubt but that the action of rain on pollen is quite as potent in preventing a set of fruit as is its injurious effect on the stigmatic fluid. Casella (2) states that in the grape, rain was found to cause the cap or calyptrum to adhere to the stigma and thus prevent pollination.

Quite as important as any of the above considerations, and possibly more so, is the fact that rain prevents the distribution of pollen by the wind and must effectually prevent bees and insects from carrying it. Weather conditions involving rain and a humid atmosphere also have much to do with the development of parasitic fungi, which may quickly destroy the blossoms or even cause a blight of whole blossom clusters. This, however, is an entirely different condition than the dropping of the blossoms as a result of incomplete fertilization.

Low temperatures and rainy weather during the blooming period may be a factor in reducing the set of the fruit in still another way. According to the investigations of Müller-Thurgau (5) on the falling of grape flowers, dull, cool weather during blossoming is especially favorable for the growth of leafy shoots which aid in the depletion of the reserve food materials that otherwise would be available for the development of the inflorescence, so that the nutrition is not sufficient for the blossoms. He states, further, however, that such a starving of the blossom cluster and, consequently, a more or less extensive shelling of the blossoms will occur also with weather favorable for blooming if abundant nitrogen is present in the soil, or if virgin soil is used for the cultivation of grapes when the luxuriant development of the vegetative organs limits the further development of the blossoms.

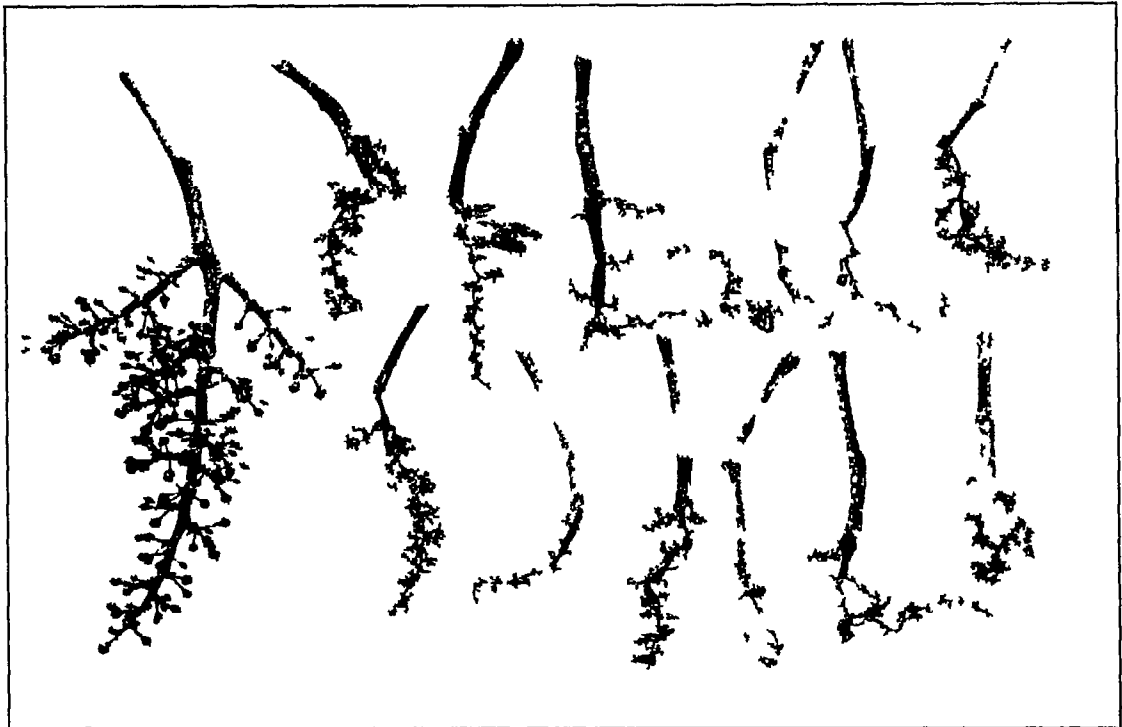
Subsequent investigations by Müller-Thurgau (6) indicate that not only long-continued cold, rainy weather, but a long period of low temperature alone, or excessive dry weather have been found conducive to

the failure of the fruit to set. It is thought by him that low temperature exercises an unfavorable influence on the metabolism of the flowers and that evaporation and the formation of sugar in the leaves are retarded, as is likewise the solution and utilization of the organic reserve material.

Shelling of the more or less fully developed berries, or late coulure, also is due to a variety of causes which come under the category of unbalanced nutrition and unfavorable climatic conditions. The agencies that contribute to shelling from these causes generally are considered to be the following: a weakening of the vines due to over-bearing, heavy vegetative growth in proportion to the root system, attack of the vine by root-rot, or to such factors as excessive nitrogen supply emphasized by over-tillage and prolonged drought or excessive rains followed by drought. It has also been held that a lack of potash is in many cases the primary cause, although there is considerable evidence that this is not a factor.

Grapes affected with shelling from purely physiological derangements engendered by these causes usually fall two or three weeks before maturity, the normally fibrous, connecting tissue of the pedicel cleaving smoothly at the point of junction with the berry. The berries at the end or at the extremity of the shoulder of the cluster, as a rule, are the first to fall. The affected berries, particularly of the green varieties, often exhibit a peculiar, though indistinct, mottling of the surface; the skin becomes abnormally thick and the whole berry is harder than normal berries of the same age. The interior of such a berry shows a brown zone immediately beneath the skin and the taste is noticeably insipid as compared to the tart, astringent flavor of the normal, unripe berries.

The shelling of mature or nearly mature grapes may also occur as the result of certain fungous diseases of the fruit, although these have been denied as the primary cause of this trouble as understood in American literature. This has been due to the fact that the term shelling has been limited hitherto to the dropping of the fruit as a result of purely physiological disorders. A typical shelling of the fruit, however, occurs when the berries are attacked by such fungi as the brown-rot form of downy mildew (*Plasmopara viticola* (B. & C.) Berl. & De Toni), the dead-arm disease (*Cryptosphaerella viticola* (Red.) Shear), the bitter-rot (*Melanconium fuligineum* (Scrib. & Viala) Cav.), the white-rot (*Coniothyrium diplodiella* (Speg.) Sacc.), and the ripe-rot (*Glomerella cingulata* (Atk.) Spauld. & v. Schrenk). Even in the case of shelling caused by these fungous diseases, the fungous disease is not always evident. During the course of the writer's investigations of grape diseases during the



NON SETTING AND BLACK ROT BLIGHT OF GRAPE FRUITS

FIG. 1 (Upper) Failure of Moore's Early grapes to set fruit is a result of the cold rainy weather that prevailed during the blooming period showing a normal cluster at the left — natural size

FIG. 2 (Lower) Blight of Concord grape blossom clusters caused by black rot showing normal cluster at the left — $\frac{1}{2}$ natural size

summers of 1922 and 1923 it was noted in vineyards at Neosho and other points in Missouri, where the grapes were attacked by bitter-rot, that many of the ripe grapes would shell off during the handling incident to picking and packing. Most of these, it is true, showed more or less conspicuous pustules, while others appeared perfectly normal to the eye, although they were infected and developed pustules after being placed in a moist chamber for a few hours or sometimes days.

Inasmuch as shelling of grapes may be caused by certain fungous diseases alone, it is evident that our usage of this term will have to be enlarged beyond the scope of miscellaneous physiological disorders, to which it has been limited previously.

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A DISEASE OF TOMATO CAUSED BY PHYTOPHTHORA MEXICANA. SP. NOV.

J. W. HOTSON AND LENA HARTGE

WITH PLATES XXII AND XXIII AND ONE FIGURE IN THE TEXT

INTRODUCTION

The genus *Phytophthora* was first named and described in 1876 by Anton de Bary (1), who was at that time making an extensive study of the disease producing potato blight. To the organism causing that disease he gave the name *Phytophthora infestans*, by which name it has since been known. Saccardo lists eight species in his *Sylloge Fungorum* (12). There are now more than twenty known species, four of which have been reported as attacking tomato. These four are as follows: tomato blight, caused by *P. infestans* (Mont.) de Bary (9); buckeye rot of tomato fruit, caused by *P. terrestris* Sherb. (13); collar rot, caused by *P. cryptogea* Pethy. (7), and the fourth, a damping off, stem-girdling *Phytophthora* as yet undescribed and unnamed (8). The present paper has to do with a fifth disease, caused, it is believed, by another species of *Phytophthora* which has been under observation and culture for more than three years. The organism herein described was isolated from some tomatoes shipped into Seattle, Washington, from Mexico in the early summer of 1917. Transfers were made of this culture from time to time to keep it alive until 1920, at which time the cultural study was begun.

The fungi of the genus *Phytophthora* were for a long time thought to be obligate parasites, but in 1900 two Frenchmen, Matruchot and Molliard, succeeded in growing *P. infestans* on artificial media. Five years later, Clinton, working at Connecticut Agricultural Experiment Station, reported somewhat similar results (2). Jones and Giddings (3), of the University of Vermont in 1909, also carried on some research work along this line with varied success. Since that time considerable cultural work has been done on this genus.

THE MYCELIUM

The mycelium of the fungus under consideration grows readily on artificial media, producing more or less dense growth on the surface of some and growing quite deeply into the substratum of others. Within twenty-four hours radiating strands of mycelium one-half inch or more long may be seen growing out from the point of inoculation. As the growth increases, if the growth be aerial in character, the medium

becomes covered with an even, white coat of mycelium, and on the other hand, if the growth be substratal in character, the natural color of the medium prevails, due to the sparse surface growth. The former is true of its growth on the lima bean agar, and the latter is characteristic of its growth on the oat agar, while on the tomato media there seems to be both aerial and deep substratal growth.

The hypha averages $7.7\ \mu$ in diameter, and is profusely branched and non-septate. In its younger stages it is full of dense, granular protoplasm, interspersed with numerous oil globules. As has been observed by other workers on the genus *Phytophthora*, the tip of the branches often burst when mounted in water and the contents ooze out in oily globules. The branching of the mycelium is very irregular. Some of the branches are of a short, knob-like character, sometimes touching and anastomosing. Where the mycelium penetrates the medium it gives the peculiar knobby appearance shown in Plate XXII, figs. 2 and 3. The knobby appearance is not a new feature, as it has been previously mentioned in the description of other species. Reddick (8), for instance, found swellings and distortions of the hyphae of a *Phytophthora* grown on beef bouillon, or beef agar with sugar. In the species under consideration, however, liquid media seem to give straight, long, slender filaments with but little of the irregularity that is characteristic of its growth on solid media. In older cultures the mycelium becomes septate, a condition noted by other workers on other species of *Phytophthora*. At this stage the filaments are usually empty, or if they contain protoplasm, it seems to have lost its continuity and to be disintegrating.

The tomato fruit, when inoculated, shows the mycelium growing in all parts of it. It seems to be intercellular in its character, appearing on the surface after about five days and within two weeks covering the entire surface with a white growth. In the stem of the tomato plant the hyphae are usually found to be intercellular, rarely intracellular, and occasionally penetrating the spiral vessels and growing up through the vascular system (Plate XXIII, fig. 15). In the leaf, roughly mounted, the mycelium may be seen through the epidermal tissue ramifying in all directions between the cells.

CONIDIOPHORES

The conidiophores (Plate XXII, fig. 11) are found in varying numbers on all cultural media, profuse on tomato agar, but rather scattering and scanty on oat agar. At different times of the year they vary greatly in abundance. In the spring and summer months they appear in great

numbers. During the past winter when conidia were desired for inoculation purposes, it was observed that they were produced very sparingly both on old and on freshly made media of various kinds. In many cases none at all were found. But with the advent of spring, conidia came again on all cultures in great numbers. This probably was a temperature condition, as the past winter was an exceptionally cold one for Seattle, and no similar conidial conditions were observed in previous years.

Cultures were made in Van Tieghem cells and the development of conidia watched. The conidiophore (Plate XXII, figs. 1, 4, 5, 6) appears first as a simple stout branch at the apex of which a single conidium is borne. The branch at the base of the conidium swells slightly, the conidium is pushed aside, and the branch elongates, producing another apical conidium. Simultaneously with this growth the primary conidium is pushed out on a long slender stalk arising by growth at its base. Often the conidia are produced so close together and so abundantly that the conidiophore assumes a cymose appearance. Rarely does the conidiophore have the swollen knob just below the branch as in *P. infestans*. But this feature is neither a common nor a typical characteristic of this species.

The conidiophores on the leaves of inoculated tomato plants may be seen pushing out through the stomata, the base always bulging noticeably just after reaching the surface of the leaf (Plate XXII, fig. 10).

THE CONIDIA

The conidia begin to form in forty-eight hours after new cultures have been made and begin to germinate immediately on reaching maturity. They vary greatly in size, ranging from 16 to 77 μ in length and from 16 to 33 μ in width. Those coming from the tomato fruit and from tomato plant are of an elongated type, often measuring twice as long as wide ($44 \times 22 \mu$ to $46.2 \times 24.2 \mu$). In the early stages of development they are filled with densely granular protoplasm surrounding a large vacuole. When the conidia reach maturity the vacuoles seem to have partially disappeared. There is also a great variety of forms, some having length and width nearly equal and others being twice as long as wide, while others are quite irregular in outline (Plate XXIII, fig. 10). All of them have exaggerated papillae through which the zoospores escape on germination of the conidia.

On several occasions the germination from beginning to its conclusion was watched. The following account is the result of observations of the

growth of the fungus in Van Tieghem cells. The first evidence of germination seemed to be the disappearance of the vacuole and slight, hardly noticeable demarcations, as if the protoplasm were dividing, and then the whole contents of the cell began to move, going about in a circle. A space appeared between the moving ball and the wall of the conidium. In this space one could discern later wave-like motions as if cilia were moving. Definite bodies could be seen rounding up and occasionally one extended farther out than the others into the space next to the cell wall (Plate XXIII, fig. 17). The motion continued for more than one hour with short intervals of rest. Then suddenly the zoospores thus formed seemed to extricate themselves from each other and darted through the papilla-like projection into the outer liquid (Plate XXII, fig. 7). This emptying process occupied only a few seconds. In no case were the zoospores seen to be liberated into a vesicle or bladder as mentioned and pictured by Rosenbaum for *P. arecae* (10).

The zoospores are oval, elongated with a slight depression on one side. No cilia were visible except in the wave-like motion when the zoospores were moving. They soon settle and round up, germinating immediately by a thick germ tube with a diameter almost equal to that of the spore itself.

Occasionally only part of the zoospores escape from the zoosporangium, the remaining ones germinating in the interior and sending out their germ tubes through the sporangial wall in different directions (Plate XXII, fig. 9). Under some conditions there seems to be little evidence of the rounding up of zoospores, but a number of germ tubes are pushed out through the papilla end of the conidium (Plate XXII, fig. 8). At other times a conidium germinates by sending out a single germ tube, often with a single conidium forming at its end (Plate XXIII, figs. 7 and 21). This conidium may germinate by a germ tube which, in turn, forms another conidium at the tip, so that as many as four successive conidia have been observed to form and germinate in a series, the last one being in a young stage full of dense protoplasm. These conidia after they have germinated being somewhat rounded and intercalary, resemble chlamydospores, but they have a distinct papilla as well as the general shape of conidia when they are formed.

CHLAMYDOSPORES

Chlamydospores, asexual intercalary spores, are sparingly produced. Only during one summer did they seem to be produced in numbers. They are of the same general appearance when mature as conidia, with

dense, granular protoplasm with many oil globules and one or two vacuoles visible. When old they become slightly yellow. On germination each produces a single germ tube, often with a conidium produced at the end of a short hypha (Plate XXIII, fig. 16). The chlamydospores are almost spherical in shape, ranging from 28 to 44 μ in diameter. The kind of media does not seem to be a factor on their production as they were found occasionally on all media used.

OOSPORES

Oospores have been found fully developed on cultures eight days old during the colder weather. Rather longer time seems to be necessary when the mycelium is producing conidia readily or when the temperature is higher. Under these conditions four weeks may elapse before the formation of oospores. At first they are about the color of the conidia, with the same granular protoplasmic contents, but without the vacuole (Plate XXIII, figs. 3, 4, 14). As they mature they become a deep yellow with a thick, rather smooth wall, which gives the appearance of having from two to three layers (Plate XXIII, figs. 2, 6, 8, 11, 13). At a still later stage the contents are often broken into larger particles. In general they are the same type of oospore as is found in *P. phaseoli*, with the antheridial cell at the base. They are nearly spherical and measure from 24 to 37.4 μ in diameter with a wall 4.4 μ thick.

The antheridium, in its first appearance, is a swollen portion of the filament and is ovoid in shape. In older cultures containing oospores the antheridium gives, almost without an exception, the appearance of being pushed up at the base as though the oogonium had penetrated it, but in its younger stage this is not the case, as it is well rounded at the base and shows no sign of pressure. In a majority of cases in this species, where the oogonium appears to emerge from the antheridium, the funnel-shaped base of the oogonium, noted by Pethybridge in his description of *P. erythroseptica*, (6) is often lacking except in very young stages of its formation. In one case where pressure was applied, the antheridium seemed to tear loose from the oospore as if it were wrapped around the stalk (Plate XXIII, figs. 19, 20).

The oogonium and the antheridium apparently come from different filaments. In one or two cases, however, branches that appeared to bear oogonia and antheridia in primordial stages, when traced back for some distance, were found to originate from the same filament but quite a distance apart (Plate XXIII, fig. 5).

Much difference of opinion has existed concerning the relation of the antheridium to the oogonium in this type of oospore. In *P. erythro-septica* Pethybridge (6) advanced the theory that the oogonial incept grew up and pierced the antheridium, growing through it and out on the other side where it appeared as a club-shaped structure and later as a spherical oogonium. This theory was borne out by Murphy (5) in morphological and cytological studies on the same species and this method of formation for *P. erythro-septica* became an established fact. A recent article by Tabor and Bunting (14) on a disease of cocoa and coffee fruits gives added weight to the above conclusions. They find that in mature oospores the separation of oospore from antheridium can be effected by pressure and the depression left indicates that the base of the oospore is within the antheridium.

In the species under observation many instances give the appearance of the antheridium having been pierced by the oogonial stalk. This is especially noticeable in older cultures. The funnel-shaped appearance of the stalk being very apparent in the younger stages (Plate XXIII, fig. 4), but almost entirely disappearing as the oospores mature (Plate XXIII, figs. 6, 8, 13).

In the later stages of the development of the oospore the stalk may be observed from the top to the bottom of the antheridium to be of equal diameter. Sometimes it is even bulged in the center, and often is thinner near the oospore than at the base. Sometimes even what appears to be a curve in the stalk may be seen opposite the middle of the antheridium. In material which contains primordial stages of the oogonium and antheridium, it is not uncommon to see the twisting of one hyphal branch about another, or the swollen knobs with another branch growing closely along its side and curving over the tip, or the mycelium forked and growing around another filament. These conditions have led to the question as to whether the oogonial incept always pierces the antheridium in its formation in this species. Might it not sometimes be formed by some other method, namely, that suggested by Clinton (2) in his work on *Phytophthora*, that is, by the oogonial branch growing up along the side of the antheridium and forming at the top? Or might it not be formed by twisting about the oogonial stalk and anastomosing as is an occasional occurrence among threads of the mycelium? For this study Van Tieghem cells were used. A thin film of media was spread on flamed cover glasses, and the fungus transferred to this with a sterile platinum needle. Conditions did not seem to be right for full develop-

ment of oospores, however, hence most of the observations were made on ordinary slides from reproducing cultures.

Many and varied experiments were used in an effort to germinate the oospores and in only a few cases did success attend the efforts. The oospore germinates with a thick germ tube with usually a swelling at the base as it emerges from the oospore. In the cases observed the oospore seemed to give rise to mycelium rather than a conidiophore as pictured and described by Rosenbaum (11) for *P. cactorum* (Plate XXIII, fig. 9).

CULTURAL STUDY

In his work on oospore production Clinton (4) tried out many kinds of media in an effort to determine the most satisfactory one for the culture of the species of *Phytophthora*. After over a thousand cultures of the downy mildews of the potato and lima bean on various nutrients, ranging from potato plugs to synthetic media, he found that the lima bean agar produced the best results.

When the cultural study of the species under consideration was begun the fungus was badly contaminated with bacteria, so the first task was to secure pure cultures. The medium first selected was the ordinary potato agar which is used successfully for the growth of most saprophytic fungi. Ordinary transfers were made with the hope that the fungus would grow more rapidly than the bacteria and that pure cultures might be obtained by transfers from the extreme edges of the growth. This method failed, as the bacteria grew as rapidly as the mycelium and followed out each hyphal thread. Weak lactic acid was then used with success. Pure cultures having been secured, media of several kinds were used for experimentation; potato, oat, lima bean, and tomato agar were used with success.

Oospores were produced in numbers on all media used, but on oat agar very few conidia were ever obtained. Two conditions were noticeable in the production of oospores, namely, the temperature and age of the mycelium. When fresh cultures were kept at a temperature of 8-10° C. they produced oospores in about eight days. They were more sparingly produced as the temperature was increased. Media made with no other ingredient than tomato juice and agar produced luxuriant growth of the fungus even when the acid condition was great enough to soften the agar. Here, too, oospores were often produced in eight days. Under milder conditions of temperature (20° C. or more) six weeks might elapse before their production.

Van Tieghem cells were used freely in the cultural study of conidio-phores and the germination of conidia. Small portions of mycelium were transferred to cover glasses and the hanging drop culture method was used. Also flamed cover glasses were spread with a thin film of media and transfers made to these for the study of the oospore. What seemed to be primordial stages in the development of oospores appeared, but for some reason never developed well. This was probably due to the lack of proper amount of oxygen or to the fact that the agar in the cell was not of sufficient depth to give the right conditions for development.

A culture of *P. parasitica* received from Dr. W. S. Beach of Pennsylvania State College, and also one of *P. terrestria* received from Dr. C. D. Sherbakoff of Knoxville, Tennessee, were cultured for comparison. The macroscopic growth of the latter is quite different on all media, growing in tufts over the surface, while the one under consideration makes a smooth growth. The former gives somewhat the tufted appearance also, but is not as noticeably different in this respect. It, however, produces largely chlamydospores with but few conidia and no oospores were observed on any of the media used.

Much work was done in an effort to germinate oospores. Van Tieghem cells with hanging drops were first made of oospores of different ages, ranging from three months to a year old. These were subjected to varying degrees of temperature without obtaining germination. Tubes were then treated with very dilute acids and transfers made to Van Tieghem cells with like results. Peroxide solution was used in tubes, then removed and transfers made. The last two tests were made with the hope that if the oospore wall were softened or if given greater amount of oxygen, the spores would germinate. Then tubes with oospores were placed in the outdoor conditions, some with sterile water added and some without, and left for one, two, three, four and eight weeks. In tubes left out under these conditions for four or eight weeks and then sterile water placed in them, when warm spring weather came, some few oospores germinated, but none of the work on the oospore germination could be considered very successful.

PROOF OF PATHOGENICITY

That the organism is pathogenic in character is shown by its prompt action on plants inoculated with the fungus having abundant conidial growth. Large numbers of young tomato plants were inoculated by

spraying with sterile water containing conidia, and when these were left under a bell jar twenty-four hours and kept reasonably moist thereafter they showed a characteristic black wilt of all parts above the inoculation (Fig 1). In some cases small pieces of agar containing the fungus were placed in the axil of leaves. Each leaf, with all parts above, gave evidence of the effect in forty-eight hours. When the fungus was placed on the soil at the base of young plants, the plants dampened off at the surface of the soil. In cases where older stems were inoculated, a much longer time (three or four days) elapsed before signs of infection appeared.

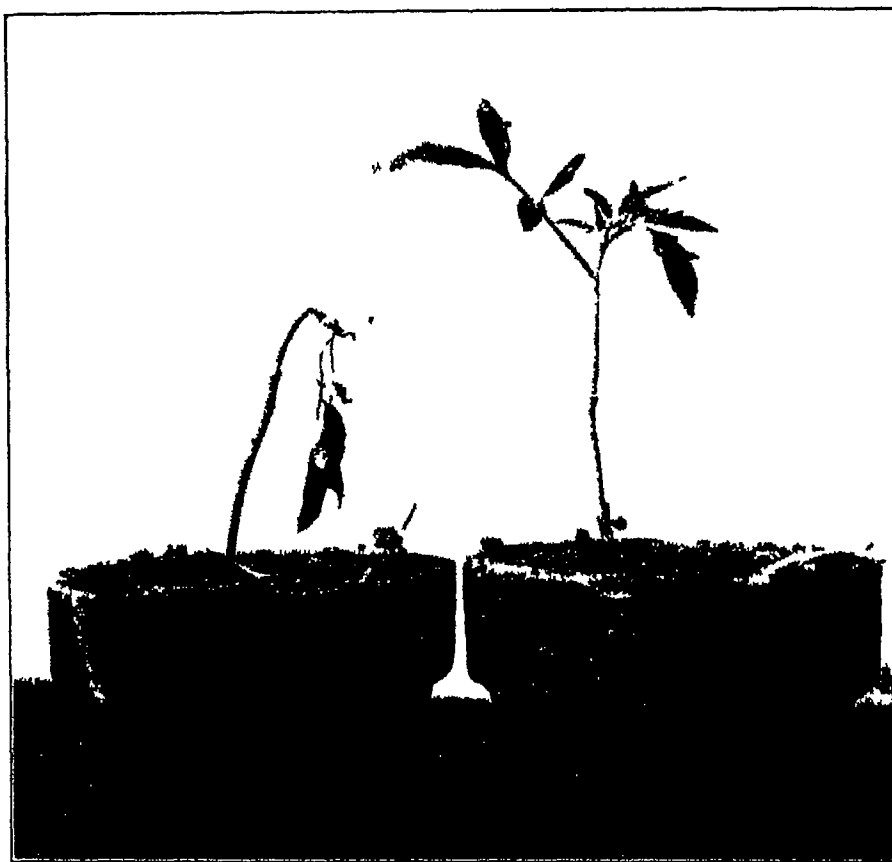


FIG. 1. The effect on the tomato plant forty-eight hours after being inoculated with *Phytophthora mexicana* Right, the control

On the tomato fruit in two days a water-soaked region appeared around the point of inoculation and in a week's time a rot had destroyed the entire fruit. The rotting in these instances did not show rings as in the disease of fruit caused by *P. terrestris*, but produced rather an even shriveling of the entire fruit by a white mycelial growth over the entire

surface. Cultures made from the center of the fruit produced luxuriant growth of the mycelium together with many conidia and oospores.

IDENTIFICATION

In comparing the fungus under consideration with known species of *Phytophthora* only those species need to be considered which have a similar position of the antheridia at the base of the oogonia. The following species show this relationship: *P. phaseoli* Thax., *P. parasitica* Dastur, *P. colocasiae* Racib., *P. infestans* (Mont.) de Bary, *P. erythro-septica* Pethyb., *P. arecae* Colem., *P. cryptogea* Pethyb., *P. terrestria* Sherb., *P. allii* K. Swadii, *P. melongenae* K. Swadii, and *P. capsici* Leon. In making this comparison *P. cryptogea* and *P. erythro-septica* can be eliminated by the lack of conspicuous conidial papillae; *P. colocasiae* by its short, blunt papillae; *P. infestans* by the scanty production of oospores; *P. arecae* by the presence of a vesicle into which the zoospores are deposited on the germination of the conidia; *P. parasitica*, *P. terrestria*, *P. allii* and *P. melongenae* by the smaller size of the oospores; *P. capsici* by the tuberos appearance produced on hard potato agar as well as the smaller sized oospores and the larger conidia; *P. phaseoli* by the smaller oospores, more variable conidia and conidiophores, as well as the fact that the oospores are not produced readily on an acid medium, while the one under consideration grows luxuriantly on media that is decidedly acid. Since the organism studied does not seem to be identical with any of the above forms, it has been described as follows as a new species.

***Phytophthora mexicana* HOTSON & HARTGE**

Mycelium irregularly branched, hyaline, non-septate when young, often septate and empty when old, sometimes short and knobby; conidiophores at first simple and bearing a single apical conidium but eventually becoming long and much branched; conidia terminal but not inhibiting the growth of the conidiophore, varying greatly in size, more or less inversely pear-shaped, 16–33 μ by 16–77 μ , sometimes measuring twice as long as wide; papillae prominent, usually forming zoosporangia; zoospores ovoid, depressed slightly on one side, germinating immediately by germ tube; chlamydospores intercalary, sparingly produced, almost spherical 28–44 μ in diameter, yellowish when old, germinating by germ tube; oospores yellowish at maturity, nearly spherical, 24–37.4 μ in diameter; episporium 4.4 μ thick; antheridium terminal or lateral, somewhat spherical or ovoid, surrounding the base of the oogonium as if penetrated by it as in *P. phaseoli* Thaxter.

Hab. On the fruit *Lycopersicum esculentum* Mill from Mexico.

Mycelio ramoso irregulariter, hyalino, non-septato recentiore sed vetustiore multi-septato vacuatoque, interdum brevi et nodoso; conidiophoris primo unicis et sustinentibus conidium unum in apice sed tandem longo ramosoque; conidiis terminalibus sine inhibitionis auctus conidiophori, variabili magnitudine, paene in forma inversi pyri, $16-33\ \mu \times 16-77\ \mu$, interdum longioribus duplo quam latioribus, papillis prominentibus, plerumque formatibus zoosporangia; zoosporis ovoidcis, depressis paulum in uno latere, germinantibus statim hyphis; chlamydo-sporis intercalaribus sed paucis, paene sphaeroideis $28-44\ \mu$ diam., luteis vetustioribus germinantibus statim hyphis; oosporis luteis vetustioribus, sphaeroideis $24-37\ \mu$ diam.; episporis $4.4\ \mu$ densis; antheridiis terminalibus et lateralibus sphaeroideis aut ovoidcis, circumambientibus bases oogoniorum quasi penetratis ab eis sic ut in *P. phaseoli* Thaxter.

Hab. In fructibus *Lycopersici exculenti* Mill, Mexico, Amer. bor.

SUMMARY

1. A fungus isolated from tomatoes shipped from Mexico proved on cultural study to be a *Phytophthora*. The mycelium averages $4.4\ \mu$ in diam., the conidia measure $16-33 \times 16-77\ \mu$. The oospores are large, the greater number of them measuring $37\ \mu$. Occasionally chlamydo-spores are produced.

2. The sexual and asexual spores have been found on all media used.

3. Inoculations of young and old tomato plants produced a black wilt. Inoculation of the fruit caused a destructive rot in a week's time.

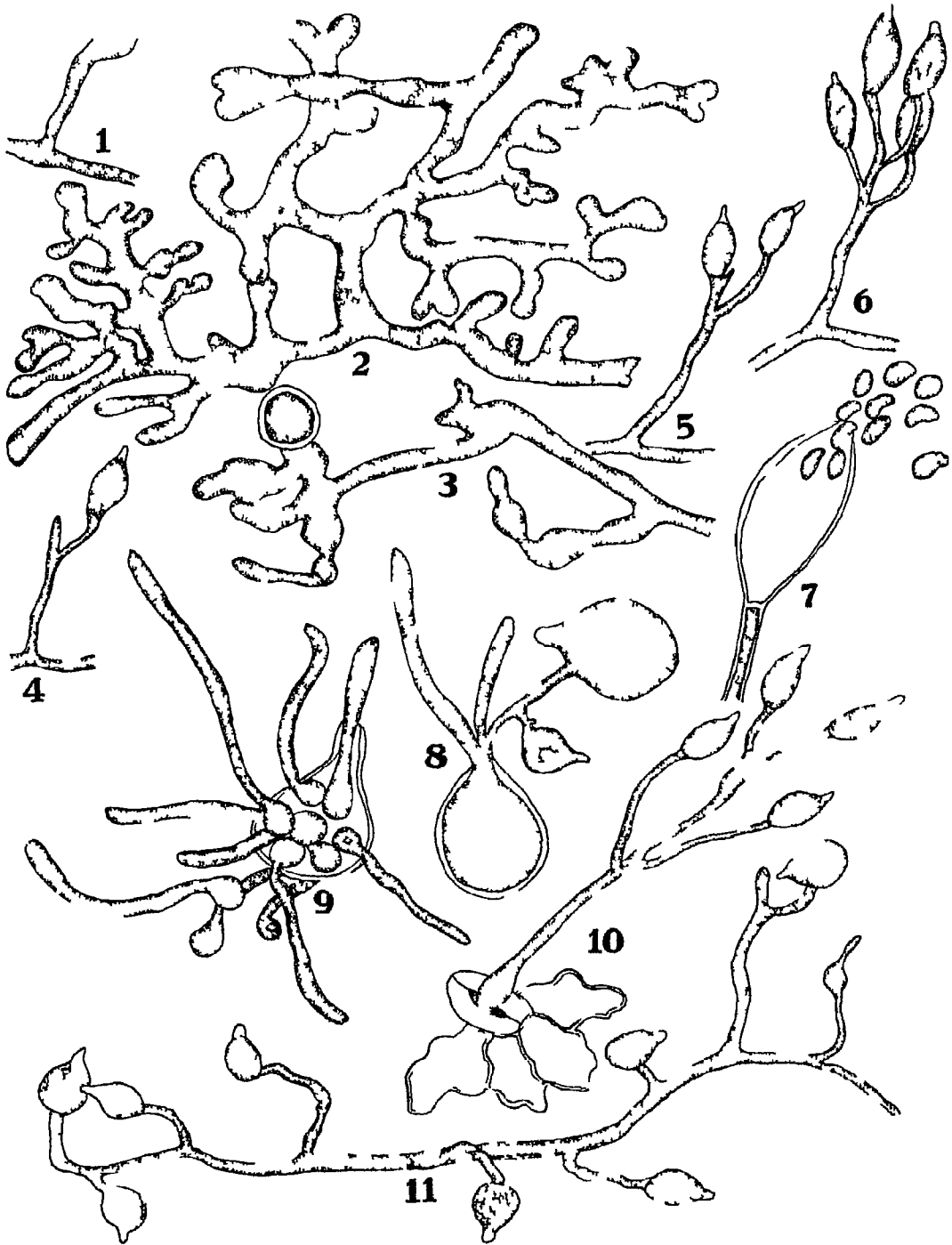
4. The organism studied does not seem to be identical with any of the known species of *Phytophthora*, and the name *Phytophthora mexicana* has been proposed.

The authors of this paper are indebted to Dr. W. S. Beach, Dr. S. D. Sherbakoff and Dr. M. W. Gardiner for cultures of *Phytophthora*.

UNIVERSITY OF WASHINGTON, SEATTLE.

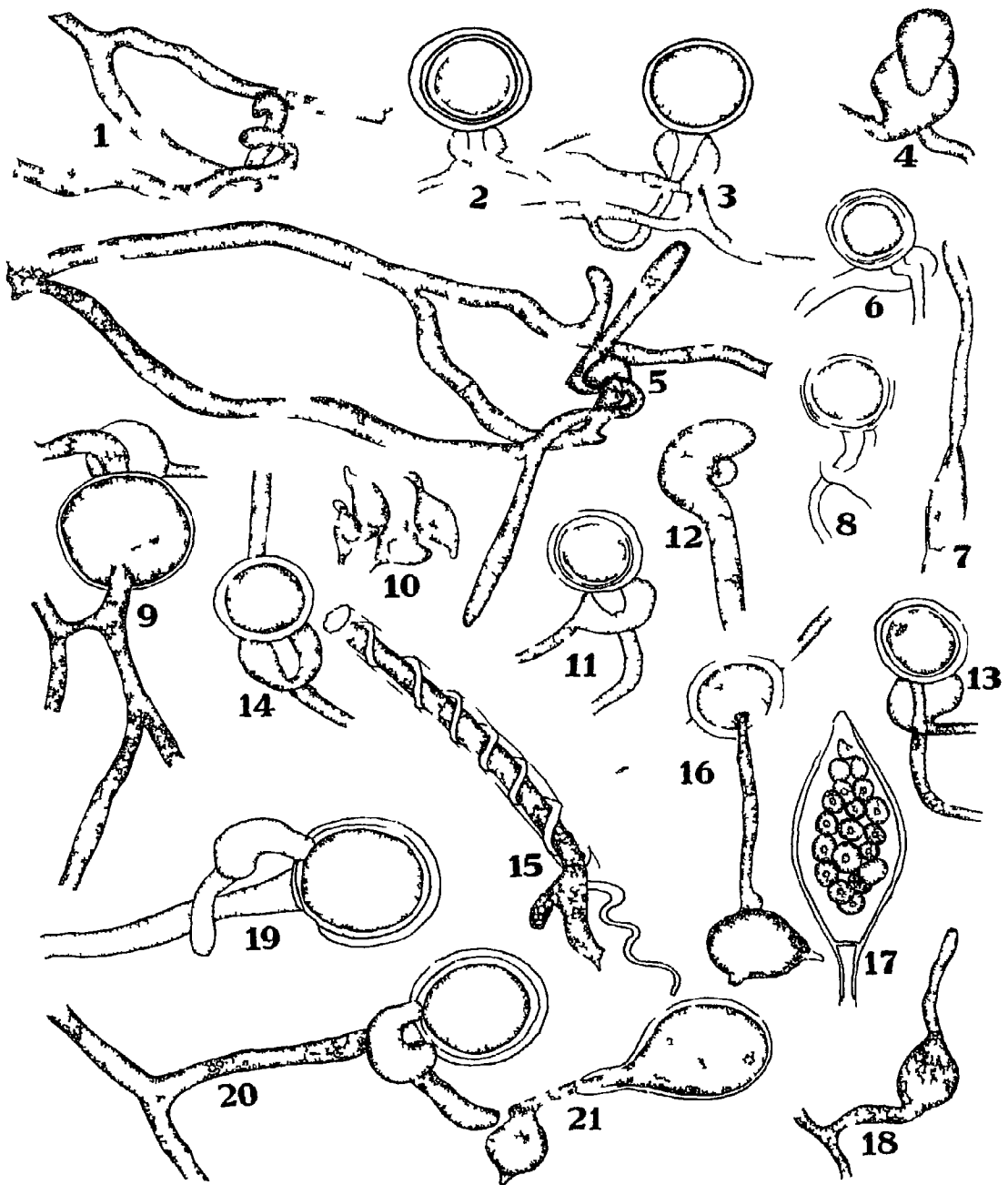
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PHYTOTHIORA MIXANA

Fig. 1—A young conidium. Fig. 2—The knobby character of the mycelium. Fig. 3—The knobby mycelium producing oospores. Fig. 4—A conidiophore showing the first conidium. Fig. 5—A young conidiophore showing two conidia. Fig. 6—A young conidiophore showing several conidia. Fig. 7—A conidium (zoosporangium) discharging zoospores. Fig. 8—A conidium germinating by sending out germ tubes. Fig. 9—A conidium showing zoospores germinating by germ tubes in the interior. Fig. 10—A conidiophore coming out through a stoma of a leaf. Fig. 11—A conidiophore showing several conidia.



PHYTOTHECA MINIMA

FIG. 1—Probably a primordial stage in the formation of an oospore and with a germ tube. FIGS. 2-4—Stages in the development of the oospore. FIG. 5—A probable primordial stage in the formation of an oospore and with a germ tube showing that the two may come from branches of the same hypha. FIGS. 6-8, 11-13-14—Oospores. FIG. 7—A conidium germinating by a single germ tube. FIG. 9—A germinating oospore. FIG. 10—Types of conidia. FIG. 12—Probably a primordial stage of an oospore and with a germ tube. FIG. 15—A hypha growing in a spore vessel. FIG. 16—A chytrid sporangium germinating by a single germ tube and immediately forming a conidium. FIG. 17—Formation of zoospores in a germinating conidium. FIG. 18—A young chytrid sporangium. FIG. 20—An oospore and with a germ tube after exerting a slight pressure. FIG. 19—The same as FIG. 20 after more pressure had been exerted. FIG. 21—A conidium germinating by a single germ tube and immediately forming another conidium.

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TOXICITY STUDIES WITH SOME COPPER FUNGICIDES

W. L. DORAN

The object of the experiments here described is to compare the susceptibility of several fungi to copper.

In the experiments described in the present paper the author has used the following fungicides: copper sulphate, neutral copper acetate, Burgundy mixture, Bordeaux mixture of various types, cuprammonium sulphate and Johnson's mixture. Spores of the following fungi were used as test objects: *Gymnosporangium clavipes*, *Gymnoconia interstitialis*, *Uromyces caryophyllinus*, *Cronartium ribicola*, *Puccinia antirrhini*, *Alternaria solani*, and *Venturia inaequalis*.

In preparing the fungicides the following methods were used: The Bordeaux mixtures, in which a weighed amount of lime was used, were made by adding the stock solution of lime to the diluted copper sulphate. Neutral Bordeaux mixture was prepared by adding to a weak solution of copper sulphate, milk of lime until a mixture alkaline to phenolphthalein was obtained. Zucker's Bordeaux mixture was made by adding saccharose to a wash containing copper sulphate and quick lime in the ratio 1:0.5 in amount equivalent to the copper sulphate taken. Perret's Bordeaux mixture was made by adding to a wash containing copper sulphate and quick lime in ratio 1:0.5 an amount of molasses equivalent to the copper sulphate used. Burgundy mixture was prepared by combining copper sulphate and sodium carbonate crystals in the ratio 1:1.84. The wash was made by adding the stock solution of sodium carbonate to a weak solution of copper sulphate. Cuprammonium sulphate (*eau celeste*) was prepared by adding ammonium hydroxide to a solution of copper sulphate in sufficient quantity to give a clear solution. Johnson's mixture was prepared according to the original formula, that is, copper sulphate and undecomposed ammonium carbonate were used in the ratio of 1:2.

The toxicity tests were made following the method of Reddick and Wallace (7), which has been described in detail by Wallace, Blodgett and Hesler (9). It consists essentially in the germination of fungous spores in drops of water on a glass surface which has been previously sprayed with the fungicide to be tested and dried. The spores were placed under optimum conditions for their germination. Clark (4) concluded that a fungus presents its greatest resistance to the action of fungicides when the temperature is at the optimum for spore germination. We cannot consider a fungicide toxic unless it is toxic under optimum

conditions for spore germination. The writer found that when the temperature was near the minimum or maximum for germination of the spores some dilutions of fungicides were toxic that were not toxic at optimum temperatures.

The results given in this paper are expressive of the point of inhibition of germination rather than of the death point of the spores. To determine the death point, requires the transfer of the spores to a fresh culture free from the fungicide. This was considered unnecessary; for Clark (3) found that, if the germination of spores is inhibited by a fungicide, they will in time be killed by it.

The data given are in each case the mean of at least five experiments, exclusive of those in which the germination of the spores in the check showed poor viability which were disregarded as untrustworthy.

In order that the copper fungicides may exert their toxicity, sufficient water must be present to act as a solvent. When the spores of *Alternaria solani* were placed in water on a slide sprayed the day before with a 1.0 per cent solution of copper acetate, the germination of the spores was prevented. But when the spores of *Alternaria solani* were placed dry on a dry slide sprayed the day before with the same concentration of copper acetate, spore germination was not prevented. They germinated as well as on a dry slide in the absence of any fungicide. In the same way, it was found that a copper sulphate solution (0.125 per cent copper) which prevents the germination of the aeciospores of *Gymnosporangium clavipes* when the spores are placed in water upon the sprayed slide, does not prevent the germination of these spores when they are placed dry upon a dry slide which has previously been sprayed with the same concentration of copper sulphate solution. Although the lethal concentration of copper sulphate solution for *Gymnosporangium clavipes* is, in the presence of water, not more than 0.125 per cent copper, it was found that a solution containing 0.5 per cent copper did not prevent germination of these spores when they were placed dry on the dried sprayed slide, so that the only available moisture was water vapor. Germination at this double concentration was, however, retarded.

EXPERIMENTS WITH COPPER SULPHATE

In table 1 are shown the results with copper sulphate. *Rhizopus nigricans* is eight times as susceptible to it as the most susceptible representative of the Uredinales and sixty-four times as susceptible as the spores of the most resistant rust fungus tested. The indications are that the Uredinales as a group are very resistant to the action of copper sulphate.

The urediniospores of *Cronartium ribicola* are somewhat more resistant to the toxic action of copper sulphate than are the aeciospores of this fungus. It appears that, while a solution containing 0.5 per cent copper sulphate might protect *Ribes* against first infection from the pine, it would require a solution containing not less than 1.0 per cent copper sulphate to protect *Ribes* from infection from *Ribes*, that is, by urediniospores. It is doubtful if these concentrations of copper sulphate could safely be used on *Ribes* foliage. Butler (1) found copper sulphate solution containing only 0.25 per cent copper sulphate toxic to the foliage of coleus, tomato, cauliflower, bean, oxalis and pelargonium.

A study of table 1 shows that the susceptibility of any one fungus to copper sulphate solutions of various concentrations is by no means uniform. The percentages of germination of all spores tested drop rapidly as the copper content increases up to 0.0039–0.0317 per cent. Beyond these concentrations of copper only the urediniospores and aeciospores of *Cronartium ribicola* and the urediniospores of *Uromyces caryophyllinus* continue to germinate in considerable numbers.

EXPERIMENTS WITH NEUTRAL COPPER ACETATE

As shown in table 2, the fungous spores tested rank as follows in their resistance to neutral copper acetate. The most resistant are the urediniospores of *Puccinia antirrhini*, and the urediniospores of *Uromyces caryophyllinus*; next comes *Alternaria solani*; while *Rhizopus nigricans* and *Botrytis cinerea* are the least resistant. Again, the Uredinales appear to be markedly resistant to copper.

TABLE 1—Toxicity of copper sulphate.

Per cent copper in solution	Relative number of spores germinating					
	<i>R. nigricans</i>	<i>G. clavipes</i>	<i>G. interstitialis</i> Aecio- spores	<i>U. caryophyllinus</i> Aecio- spores	<i>C. ribicola</i> Uredinio- spores	<i>C. ribicola</i> Uredinio- spores
0.0000	100	100	100	100	100	100
0.0039	6	.	.	..	..	...
0.0079	0	15	.	69	25	..
0.0158	0	..	.	29	20	..
0.0317	0	0	6	5	20	15
0.0635	0	0	0	2	7	9
0.127	0	0	0	1	0	3
0.254	0	0	0	0	0	0

TABLE 2—*Toxicity of neutral copper acetate.*

Per cent copper in solution	Relative number of spores germinating				
	<i>P. antirrhini</i>	<i>U. caryophyllinus</i>	<i>A. solani</i>	<i>R. nigricans</i>	<i>B. cinerea</i>
0 0000	100	100	100	100	100
0 0049					0
0 0099			16 5	0	0
0 0199			11	0	0
0 0398	3	3	3	0	0
0 0796	2	3	0	0	0
0.1592	0	0	0		0
0.3185	0	0	0		0
0.6370		0			

EXPERIMENTS WITH BURGUNDY MIXTURE

The fungous spores used in the experiments with Burgundy mixture show the following degree of resistance, the most resistant named first: urediniospores of *Cronartium ribicola*, urediniospores of *Puccinia antirrhini*; *Alternaria solani*, aeciospores of *Cronartium ribicola*, aeciospores of *Gymnoconia interstitialis*, and conidia of *Venturia inaequalis*. The results are given in table 3.

Attention is here called to the extreme susceptibility to copper in Burgundy mixture of *V. inaequalis*. That this is the normal behavior of this fungus against copper will appear subsequently. A Burgundy mixture containing only one-eighth pound of copper sulphate in fifty gallons entirely prevents germination of the conidia of this fungus.

Here, as in the case with copper sulphate, the urediniospores of *C. ribicola* are more resistant to the action of the fungicide than are the aeciospores of this fungus. A concentration of Burgundy mixture sufficient to protect the host against uredinial infection by this fungus would probably be dangerous to the host plant.

Of the representatives of the Uredinales studied, the aeciospores of *G. interstitialis* are the most susceptible to the copper in Burgundy mixture. This would perhaps make possible the control of orange rust of raspberries by spraying with Burgundy mixture if it were not for the perennial habit of the mycelium in the cane.

Burgundy mixture shows up decidedly better against *P. antirrhini* than does Bordeaux mixture. Doran (5) has already shown that Bordeaux mixture does not prevent the germination of the spores of this fungus even when 1.0 per cent copper is present.

TABLE 3—*Toxicity of Burgundy mixture 1:1.84.*

Per cent copper in solution	Relative number of spores germinating					
	<i>V. inaequalis</i>	<i>A. solani</i>	<i>G. interstitialis</i>	<i>C. ribicola</i> Aecio-spores	<i>C. ribicola</i> Uredinio-spores	<i>P. antarrhini</i>
0.0000	100	100	100	100	100	100
0.0079	0	..	...	.	...	...
0.0158	0	..	...	..	...	...
0.0317	0	62	5	..	..	...
0.0635	0	35	0	9	79	57
0.1270	0	10	0	0	35	21
0.2540	0	0	0	0	11	6
0.5080					0	0

TABLE 4—*Toxicity of Bordeaux mixture 1: 1.*

Per cent copper in solution	Relative number of spores germinating			
	<i>A. solani</i>	<i>V. inaequalis</i>	<i>G. clavipes</i>	<i>C. ribicola</i>
0.0000	100	100	100	100
0.0038	..	6	...	...
0.0079	..	2	...	...
0.0158	..	0	...	...
0.0317	81	0	...	...
0.0635	63	0	20	29
0.127	14	0	19	21
0.254	1	0	17	10
0.508	0	0	7	10

TABLE 5—*Toxicity of Zucker's Bordeaux mixture to the conidia of V. inaequalis.*

Per cent copper in solution	Relative number of spores germinating
0.0000	100
0.0019	4
0.0039	0
0.0079	0
0.0158	0
0.0317	0
0.0635	0
0.127	0
0.254	0

EXPERIMENTS WITH BORDEAUX MIXTURE 1:1

The results with Bordeaux mixture are summarized in table 4. Bordeaux mixture is toxic to the spores of *Alternaria solani* when 0.508 per cent copper is present. It is toxic to the conidia of *Venturia inaequalis* when as little as 0.0079 per cent copper is present. It is not toxic to the aeciospores of *Gymnosporangium clavipes* nor to the urediniospores of *Cronartium ribicola* when as much as 0.508 per cent copper is present.

Of these four fungi, *Venturia inaequalis* is the most susceptible and *Cronartium ribicola* is the most resistant to the fungicidal action of Bordeaux mixture. It is evident that the dissemination of *Cronartium ribicola* from currant to currant cannot be prevented by spraying with Bordeaux mixture even when this is prepared to contain twice the usual amount of copper. Unit for unit of copper, Burgundy mixture is more toxic than is Bordeaux mixture to the urediniospores of *C. ribicola*.

EXPERIMENTS WITH BORDEAUX MIXTURES 1:5 AND 1:3.3

Tests were made of the toxicity to the conidia of *Venturia inaequalis* of the Bordeaux mixture described by Sanders and Brittain (8). Two Bordeaux mixtures were used, one made up at the rate of two pounds, and the other at the rate of three pounds of copper sulphate and ten pounds of lime in forty gallons of water. Bordeaux mixtures 1:5 and 1:3.3 have a fungicidal efficiency against this fungus which is at least equal to that of ordinary Bordeaux mixture.

EXPERIMENTS WITH ZUCKER'S BORDEAUX MIXTURE

In table 5 are given the results of the tests of the toxicity of Zucker's sugar-Bordeaux mixture to the conidia of *Venturia inaequalis*. The mixture is toxic to these spores when as little as 0.0039 per cent copper is present. Therefore, the toxicity of Bordeaux mixture 1:0.5 to the conidia of *V. inaequalis* is increased four times by the addition of sugar.

TABLE 6—Toxicity of Perret's Bordeaux mixture to the conidia of *V. inaequalis*.

Per cent copper in solution	Relative number of spores germinating
0.0000	100
0.0019	5
0.0039	1
0.0079	0
0.0158	0
0.0317	0
0 0635	0
0 127	0
0 254	0

TABLE 7—*Toxicity of cuprammonium sulphate.*

Per cent copper in solution	Relative number of spores germinating		
	<i>C. ribicola</i>	<i>U. caryophyllinus</i>	<i>B. cinerea</i>
0.0000	100	100	100
0.0079	..	...	...
0.0158	...	75	0
0.0317	..	56	0
0.0635	6	36	0
0.1270	0	32	0
0.2540	0	10	0

EXPERIMENTS WITH PERRET'S BORDEAUX MIXTURE

The results of the tests of the toxicity of Perret's Bordeaux mixture to the conidia of *Venturia inaequalis* are shown in table 6. It is twice as toxic to these spores as is Bordeaux mixture 1:1, being intermediate in this respect between Bordeaux mixture 1:1 and Zucker's Bordeaux mixture.

EXPERIMENTS WITH CUPRAMMONIUM SULPHATE

The results of the toxicity tests with cuprammonium sulphate are shown in table 7. *Botrytis cinerea* is most susceptible to this fungicide, the germination of its spores being prevented when as little as 0.0079 per cent copper is present. The germination of the aeciospores of *Cronartium ribicola* is prevented by cuprammonium sulphate containing 0.127 per cent copper. This strength would possibly be toxic to the foliage of the currant, for Butler (1) found cuprammonium sulphate of this concentration to be toxic to coleus and bean. The germination of the urediniospores of *Uromyces caryophyllinus* is not prevented even when 0.254 per cent copper is present, and the use of cuprammonium sulphate for the control of carnation rust is evidently out of the question.

When cuprammonium sulphate dries on a sprayed surface there is deposited, according to Butler (1) a basic sulphate of copper and mixed with it there is a little ammonium sulphate in the ratio of 1:0.35.

What part, if any, of the toxicity of cuprammonium sulphate is due to the above mentioned ammonium sulphate was next determined. Solutions of ammonium sulphate containing from 0.0312 to 2.0 per cent of the salt were found to be non-toxic to the spores of *Botrytis cinerea*. Solutions of these concentrations did not prevent germination of the

urediniospores of *Uromyces caryophyllinus*, although the most concentrated reduced their germination 40 per cent. Evidently ammonium sulphate is not itself alone, toxic at the strength at which it is found in cuprammonium sulphate.

TABLE 8—*Toxicity of Johnson's mixture.*

Per cent copper in solution	Relative number of spores germinating			
	<i>P. antirrhini</i>	<i>U. caryophyllinus</i>	<i>C. ribicola</i> urediniospores	<i>C. ribicola</i> aeciospores
0 0000	100	100	100	100
0 0317			.	6
0 0635	45	9	4	0
0 1270	9	3	0	0
0 2540	0	0	0	0

EXPERIMENTS WITH JOHNSON'S MIXTURE

As is shown in table 8, the fungous spores tested rank as follows as regards their resistance to Johnson's mixture (6), the most resistant being named first; urediniospores of *Puccinia antirrhini*, urediniospores of *Uromyces caryophyllinus*, urediniospores of *Cronartium ribicola*, and aeciospores of *C. ribicola*. The good showing made by Johnson's mixture may be due in part to the ammonium sulphate present. Butler (1) found that the presence of ammonium sulphate increases the toxicity of a copper wash to *Plasmopara viticola*.

RELATIVE SUSCEPTIBILITY OF FUNGI TO COPPER

We are now in a position to determine how some of the pathogenic fungi compare in susceptibility to copper fungicides. Clark (3) did pioneer work along this line; and of the fungi with which he worked those found to be most resistant to copper sulphate were the molds *Aspergillus*, *Sterigmatocystis*, *Botrytis*, and *Penicillium*. Toward the fungicides with which Clark (2) worked, the fungi studied proved increasingly resistant in the following order: *Botrytis*, *Oedocephalum*, *Penicillium* and *Aspergillus*.

The results obtained by the writer are sufficient to make possible a comparison of the susceptibility to copper fungicides of aeciospores and urediniospores of Uredinales. These are summarized in table 9, a study of which shows that the average lethal concentration in per cent copper

for aeciospores is 0.0834 and for urediniospores it is 0.2746. In other words, it requires 3.2 times as much copper to prevent the germination of urediniospores as it does to prevent the germination of aeciospores. It is probable that no disease of plants dependent upon urediniospores for its dissemination can be successfully combatted by the use of copper fungicides, since sufficient copper to check the fungus would also injure the host plant.

The average lethal concentration for all spores of Uredinales was 0.179 per cent copper as compared with a lethal concentration of 0.088 per cent copper for all other spores. The spores of Uredinales are twice as resistant to copper as the spores of other fungi.

The order of resistance to copper fungicides is as follows with copper sulphate: urediniospores of *C. ribicola*, aeciospores of *C. ribicola*, urediniospores of *U. caryophyllinus*, aeciospores of *C. ribicola*, aeciospores of *G. interstitialis*, aeciospores of *G. clavipes*, spores of *R. nigricans*; with Bordeaux mixture: urediniospores of *C. ribicola*, aeciospores of *G. clavipes*, spores of *A. solani*, conidia of *V. inaequalis*; with copper acetate: urediniospores of *P. antirrhini*, (equal to) urediniospores of *U. caryophyllinus*, spores of *A. solani*, *R. nigricans*, *B. cinerea*; with cuprammonium sulphate: urediniospores of *U. caryophyllinus*, aeciospores of *C. ribicola*, uredinio-

TABLE 9—*Susceptibility of aeciospores and urediniospores to copper.*

Fungus	Fungicide	Lethal concentration	
		Aeciospores	Urediniospores
		Per cent copper	Per cent copper
<i>G. clavipes</i>	Copper sulphate	0.0317	
<i>G. interstitialis</i>	Copper sulphate	0.0635	
<i>U. caryophyllinus</i>	Copper sulphate		0.2540
<i>C. ribicola</i>	Copper sulphate	0.1270	0.2540
<i>U. caryophyllinus</i>	Copper carbonate		1.1480
<i>P. antirrhini</i>	Copper acetate		0.1592
<i>U. caryophyllinus</i>	Copper acetate		0.1592
<i>G. interstitialis</i>	Burgundy mixture	0.0570	
<i>C. ribicola</i>	Burgundy mixture	0.1140	0.4580
<i>P. antirrhini</i>	Burgundy mixture		0.4580
<i>P. antirrhini</i>	Johnson's mixture		0.2540
<i>C. ribicola</i>	Johnson's mixture	0.0635	0.1270
<i>U. caryophyllinus</i>	Johnson's mixture		0.2540
<i>C. ribicola</i>	Cuprammonium sulphate	0.1270	
<i>U. caryophyllinus</i>	Cuprammonium sulphate		0.5000

spores of *P. antirrhini*, spores of *B. cinerea*; with Johnson's mixture: urediniospores of *U. caryophyllinus*, (equal to) urediniospores of *P. antirrhini*, urediniospores of *C. ribicola*, aeciospores of *C. ribicola*.

The average amount of copper necessary to prevent germination of the conidia of *V. inaequalis* is 0.0145 per cent as compared with an average of 0.133 per cent for all other spores. The apple scab fungus is about nine times as sensitive to copper as the average of the other spores tested.

SUMMARY

The Uredinales are twice as resistant to the toxic action of copper as are other fungi. Urediniospores of these fungi are three times as resistant to copper as are aeciospores. Spraying with copper fungicides for the prevention of infection by urediniospores is probably impracticable. The indications are that Johnson's mixture will succeed against urediniospores if any copper fungicide will.

The conidia of *V. inaequalis* are extremely susceptible to copper in any form, being nine times as sensitive to it as the average of all other spores studied.

Bordeaux mixtures of the 2-10-40 and 3-10-40 formulae were not found to differ from Bordeaux mixture of the 4-4-50 formula in toxicity to the conidia of *Venturia inaequalis*.

By the addition of sugar, at the rate of 4 pounds in 50 gallons of 4-2-50 Bordeaux mixture, the toxicity of the wash to the conidia of *Venturia inaequalis* is increased four times.

Bordeaux mixture of Perret's formula, prepared by the addition of molasses, is twice as toxic as is ordinary Bordeaux mixture to the conidia of *Venturia inaequalis*.

Aeciospores of *G. clavipes* are so resistant to the copper in Bordeaux mixture that spraying the conifer host of this, and presumably other Gymnosporangiums, with this fungicide would prove of doubtful value.

Burgundy mixture surpasses Bordeaux mixture in its toxicity to the spores of *A. solani*.

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THE PRODUCTION BY *FUSARIUM SOLANI* OF A TOXIC EXCRETORY SUBSTANCE CAPABLE OF CAUSING WILTING IN PLANTS

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Many plants, such as cotton, potatoes, banana trees, carnations and others are liable to be attacked by *Fusarium*. The parasite seems to gain entrance through the root system, developing mainly in the vascular tissue.

According to some authors² the fungal hyphae are supposed to cause a mechanical plugging of the vessels, and bring about the wilting of the host plant by cutting off its water supply; or as MacMillan³ puts it: "That the fungus has created a physiological drouth which has extended over a long period."

Butler⁴ states that in the case of pigeon pea wilt caused by *Fusarium udum*: "Though the water supply to the roots may be ample, its passage to the green parts is impeded by the choking of the vessels; this may be sufficient to produce the same effect as severe drought, but it is possible that poisonous substances excreted by the fungus may also play an important part."

It has also been stated, in the case of *F. cubensie*, causing banana wilt,⁵ that the fungus tissue in the vessels is not present in quantity sufficient to cause serious obstruction to the passage of water.

It appears that the blocking by the fungus of the vascular tissue of the host to any serious extent is doubtful; and the presence of some factor other than the "mechanical plugging" is highly probable. This factor, responsible for the wilting, may be in the nature of diffusible substances injuring the living plant, and causing it to wilt. Such substances may be enzymes or other excretory products produced by the fungus during its growth in the plant.

¹The work here published was done in 1920 at the Imperial College of Science, London.

²Masse, G. Diseases of cultivated plants and trees pp. 490-496. Edition 1915.

³MacMillan, H. G. *Fusarium* blight of potatoes under irrigation. Jour. Agric. Research. 16: 279-304. 1919.

⁴Butler, E. J. Fungi and diseases in plants. p. 247. Edition 1918.

⁵Brandes, E. W. Banana wilt. Phytopathology 9: 339-390 1919.

The object of the present investigation was to find out whether or not;

(1)—Fusarium, such as *Fusarium solani*, produces toxic substances capable of causing wilting in plants.

(2)—These substances, if present, are of enzymic nature.

THE FUNGUS

A slant of potato agar¹ was inoculated with *F. solani* from a stock culture.² This slant was then incubated at 25° C. for 24 days after which it was examined and had the following characters.

Fusiform macroconidia and globose chlamydospores present, of the following measurements:—

0—Septate	(8–10) × (4–5) ¹ / ₂ μ
1—Septate	(18–24) × (5–6) ¹ / ₂ μ
2—Septate	(26–28) × (5–6.5) μ
3—Septate	(28–36) × (6.5–7.5) μ
4—Septate	(40–47) × (6.7–8) μ
very few	
Chlamydospores	6 × 8 μ

The aerial mycelium, well developed, olive white in color, substratum buff rather than olive.

A dilute spore suspension was made in sterile water, and a drop of this was placed in the centre of a sterile petri-dish, by lifting its cover just enough to permit the drop to be deposited, the petri-dish was then inverted and the drop examined under the microscope with the $\frac{2}{3}$ objective. Nine spores were found present in this drop; melted agar at 40° C. was poured into the petri dish, and incubated at 25° C. for three days, after which it was examined. Nine centres of growth were apparent to the naked eye; one of these was transplanted on to a sterile petri-dish containing sterile potato agar, and incubated at 25° C. for 24 days. When examined it was found to be similar in all its characters to the former culture.

In this way a pure culture of *F. solani* raised from a single spore was obtained, one strain only being present.

¹ The potato agar was prepared in the following way:—a)—400 grms. of peeled potatoes were steamed for an hour with a little water, then passed through cheese cloth. b)—15 grms. of agar in 500 ccs. of water were cooked at 110° for 30 minutes; to this the potato was added and the whole made up to 1 litre, well mixed, tubed and sterilized by intermittent heat, to prevent the potato from darkening.

² The original culture was obtained from Dr. Horne

THE EXPERIMENT

The media. The first thing required was a liquid medium on which the fungus could grow freely, and when in a pure sterile state would not bring about wilting of stems, such as those of broad bean plants, placed in it.

Turnip juice medium. Turnip juice was first tried as medium: 400 grams of white peeled turnips were cut up into small pieces, placed in a flask, plugged, and, without the addition of any water steamed for two hours and a half; the juice was then extracted without the addition of any water, and 240 ccs. of pure turnip juice were obtained. Solutions were made up containing the following percentage of the pure turnip juice:—24 per cent, 20 per cent, 15 per cent, 12½ per cent, and 5 per cent. All were autoclaved for 20 minutes at 120° C.

The fungus grew well on all these solutions, but when stems of broad bean four weeks old were placed in the different solutions of the turnip juice, wilting occurred within a few hours in the two stronger solutions, namely the 24 per cent and the 20 per cent. The wilting of the bean stems occurred earlier and was more pronounced than that of similar bean stems placed in the filtrate of a culture of the fungus after two weeks growth. For this reason the turnip juice as a medium was abandoned.

Richard's solution. The next medium to be tried was Richards Solution, the composition of which is:—

Potassium Nitrate (KNO ₃)	10 grms.
Potassium Hydrogen phosphate (KH ₂ PO ₄)	5 grms.
Magnesium sulphate (MgSO ₄)	2.5 grms.
Iron Chloride (FeCl ₃)	20 mgrms.
Cane Sugar (C ₁₂ H ₂₂ O ₁₁)	50 grms.
Distilled water	1,000 cc.

These quantities were dissolved and autoclaved.

The fungus grew very well on this medium, in fact much better than it did on turnip juice medium, but the pure sterile Richards' solution produced wilting of broad bean within 5–6 hours. To avoid this effect the medium was diluted and it was found that by using 1,500 cc. of water instead of the original 1,000 cc., as given in Eyres' bacteriological technique, wilting was entirely avoided; in fact broad beans placed in such a medium remained fresh for 15 days and even showed marked growth. The fungus grew equally well on this dilute medium, which, being the most satisfactory, was chosen to grow the fungus for this investigation.

Growing the fungus. A number of 500 cc. conical flasks, each containing 200 cc. of Richards' solution were autoclaved for 20 minutes at 120° C. When cold they were inoculated with the mycelium of the fungus grown on potato agar,¹ and incubated at 25° C. A week later an equal number of similar flasks was inoculated and incubated at the same temperature, and still a week later the operation was repeated. After a period of three weeks from the first lot of inoculations, the flasks were taken out of the incubator and each lot separately filtered under sterile conditions. In this way the filtrates obtained were of cultures one week, two weeks and three weeks old.

In each case the filtrate was divided into two portions; one was boiled for a few seconds, and when cold, sterile air was passed through it to replace that which was lost during the boiling. The other part was left unboiled. The object of the boiling was to destroy any thermolabile substances such as enzymes which may have been present, leaving only those which are thermostable.

Solutions of half concentration were made from the boiled and unboiled filtrate by diluting them with distilled water.

A number of test tubes were filled equally with the different solutions, others with distilled water, and others with sterile Richards' solution—the two latter types to serve as controls. In each of these tubes a stem of broad beans was inserted. Besides the water and Richards' solution used as control, bean stems were inserted in empty test tubes. In all cases the bean stems were plugged round with cotton wool.

The broad beans used were grown for three weeks in the greenhouse after being germinated, when used they were 5 inches long and had three pairs of well developed leaves.

The different test tubes with the bean stems were placed in the greenhouse at about 11 a. m. in the morning. The temperature during the months of April and May—during which time the experiment was repeated six times with the same result—was between 60° and 70° F.

The time required for the first signs of wilting to be apparent differed slightly with the temperature of the greenhouse.

The following are the maximum and minimum time required for broad bean stems to wilt in the different filtrates:—

Filtrate	1 week old	2 weeks old	3 weeks old
Boiled Filtrate	20-24 hours	4-6 hours	2-4 hours
Unboiled Filtrate.	20-30 "	4-8 "	2-4 "
Boiled Fte. $\frac{1}{2}$ strength.	40-48 "	6-10 "	4-6 "
Unboiled Fte. $\frac{1}{2}$ strength	40-50 "	6-10 "	4-6 "

¹ The fungus was grown from the single spore culture obtained on potato agar plates.

Controls

Pure Richards' solution	12-15 days
Distilled water solution	3- 7 days
Air	1- 3 hours

Though the effect of the boiled and unboiled filtrate appeared at about the same time, the bean stems in the unboiled showed the first signs of blackening at the cut end, while often the very earliest signs of wilting appeared in the boiled filtrate. Wilting due to the filtrate, whether boiled or unboiled, appeared first in the two lower leaves, the upper ones remaining apparently fresh, though for a short time only; they soon wilted in a similar manner. The bean stems in the empty test tubes collapsed, wilting uniformly in all the leaves. If, however, these were placed in water even after very marked wilting, they recovered, while those in filtrates never recovered after such treatment.

The filtrate. The filtrate was a clear liquid varying from light to darker shades of amber according to the age of the culture. It had no disagreeable smell and was distinctly alkaline in reaction. When boiled with soda lime, free ammonia was given off.

The following are the average results obtained when the filtrate was titrated with $\text{HCl } \frac{N}{10}$, using sodium alizarine sulphate as indicator:—

Filtrate of culture 1 week old: 20 cc. = 3 cc. of $\frac{N}{10}$ HCl or an alkalinity of - 15
(Fuller's)¹

The - 15 for example means that exactly 15 cubic centimeters of normal acid N/1 must be added to a litre of the substance to render it neutral to the indicator used, and correspondingly + 24 means that the substance is acid and that 24 cubic centimeters of normal alkali N/1 would be needed to be added to the litre to bring it to the neutral point.

Filtrate of culture 2 weeks old: 20 cc. = 12 cc. $\frac{N}{10}$ HCl
or an alkalinity of - 60

Filtrate of culture 3 weeks old: 20 cc. = 16 cc. $\frac{N}{10}$ HCl
or an alkalinity of - 80

The Richards' solution had, in the pure sterile state, an acid reaction:

20 cc. of Richards' solution = 4.8 cc. $\frac{N}{10}$ NaOH or an acidity of + 24.

From this it can be easily seen that the growth of the fungus rendered the medium alkaline, the degree of which increased with the age of the culture, as follows:—

Alkalinity due to one week's growth:	24 + 15	or	39
“ “ two “ “	24 + 60	or	84
“ “ three “ “	24 + 80	or	104

¹ The plus and minus on Fuller's scale denote respectively acidity and alkalinity of the substance.

The effect of the alkalinity of the filtrate on wilting. It was desirable to find out whether the alkalinity of the filtrate was the cause of the wilting.

Solutions of -15, -60 and -80 NaOH and NH_4OH (spec. gr. .88) were made up and compared with the filtrates of cultures one week, two weeks and three weeks old respectively as to their wilting power on broad beans.

The results were as follows:—

<i>Solution</i>	<i>Time for the first signs of wilting to appear</i>	
-15 NaOH.....	5.5	days
- NH_4OH ..	5	"
Filtrate of culture 1 week old having alkalinity of -15 ...	18.5	hours
-60 NaOH.....	4.5	days
-60 NH_4OH ..	4	"
Filtrate of culture 2 weeks old having alkalinity of -60.....	4 5	hours
-80 NaOH	3	days
-80 NH_4OH	3	"
Filtrate of culture 3 weeks old having alkalinity of -80.....	2.5	hours

The wilting in the different alkaline solutions, namely the NaOH and NH_4OH , was uniform in all the leaves when it became apparent. The cut ends of the bean stems were black in colour in the stronger solutions: namely the -60 and the -80 NaOH and NH_4OH at the time when the wilting became apparent.

It was obvious from the preceding experiment that the alkalinity of the filtrate was not responsible for the wilting produced; moreover when filtrates of cultures from one to three weeks old were either neutralized or rendered acid to the same degree as the original medium, namely the +24, wilting occurred as in the untreated filtrate. Therefore it can be said that though an alkaline reaction develops progressively with the age of the culture, this reaction is not directly responsible for the wilting of the bean stems.

Volatile substances in filtrate. Known amounts of filtrates of different ages were placed in an oven at 100°C . for an hour to allow any volatile substance present in the filtrate to escape. When cooled down to the same temperature as the unheated, distilled water was added to replace that which was lost due to the heating. These were compared with the unheated filtrate of the same age, but showed no loss of their wilting power. It can be said that the factor responsible for the wilting is not a volatile substance.

An oxalate present in the filtrate. Filtrates of three weeks old cultures were tested for an oxalate by the following methods:—

1)—A portion of the filtrate was heated with concentrated sulphuric acid, CO_2 was given off and its presence was detected by its action on lime water.

2)—Also, a portion of the filtrate was neutralized and a solution of calcium chloride was added to it; a white precipitate of calcium oxalate was formed which was soluble in dilute hydrochloric and nitric acids but insoluble in acetic acid.

3)—Also an acid solution of potassium permanganate was added drop by drop to a portion of the filtrate; the colour of the permanganate was discharged, owing to the reduction by the oxalic acid. It is clear that an oxalate is present in the filtrate.

Estimation of the acid radical C_2O_4 in the filtrate of a three weeks old culture: To 50 cc. of the filtrate, a solution of CaCl_2 was added, and the precipitate formed was dissolved in dilute HCl and made up to 50 cc.

When titrated with KMnO_4 , it gave the following average result:—

$$\begin{aligned} 20 \text{ cc. } \left(\frac{\text{N}}{10}\right) \text{ of filtrate} &= 4.5 \text{ cc. } \left(\frac{\text{N}}{10}\right) \text{ KMnO}_4 \\ 1 \text{ cc. } \left(\frac{\text{N}}{10}\right) \text{ C}_2\text{O}_4 \text{ " } &= 1 \text{ cc. } \left(\frac{\text{N}}{10}\right) \text{ KMnO}_4 \\ 1 \text{ cc. } \left(\frac{\text{N}}{10}\right) \text{ C}_2\text{O}_4 \text{ contains } &\frac{(12 \times 2) + (16 \times 4)}{2 \times 10 \times 1000} \text{ or } .0044 \text{ gm. of C}_2\text{O}_4, \text{ i. e., } 4.5 \text{ cc.} \end{aligned}$$

$\left(\frac{\text{N}}{10}\right) \text{ K M n O}_4 = .0044 \times 4.5 \text{ or } .0198 \text{ gm. of C}_2\text{O}_4 \text{ or } 20 \text{ cc. of filtrate contains } .0198 \text{ gm. of C}_2\text{O}_4; \therefore \text{ percentage is } .099 \text{ of C}_2\text{O}_4 \text{ in filtrate.}$

The wilting power of different solutions of C_2O_4 . Solution of $(\text{NH}_4)\text{C}_2\text{O}_4$ containing .099 per cent C_2O_4 , or of a strength of $\frac{\text{N}}{40} \text{ C}_2\text{O}_4$ in a litre of distilled water; this solution failed to produce wilting when freshly cut bean stems were placed in it. Even after three days the bean stems were still fresh and healthy.

Similarly a solution of $\frac{\text{N}}{10} (\text{NH}_4)_2 \text{C}_2\text{O}_4$ was tried but did not produce wilting except after three days, while a solution of $\frac{\text{N}}{1}$ produced wilting in three hours. In the $\frac{\text{N}}{1}$ solution 4.4 per cent of C_2O_4 was present; while the filtrate of a three weeks old culture, which produced wilting in about the same time as the $\frac{\text{N}}{1}$ solution, contained only .099 per cent of C_2O_4 .

Though the wilting in both was similar in appearance, it cannot be said that the oxalate present in the filtrate is directly responsible for the wilting.

CONCLUSIONS

(1) *Fusarium Solani*, when grown under artificial conditions, produces a toxic diffusible substance which increases in concentration with the age of the culture. This toxic substance produces a rapid wilting of cut bean stems when these are placed in the filtrate of the fungus.

(2) The culture becomes alkaline due to the growth of the fungus. The degree of alkalinity increases with the age of the culture; this alkalinity, however, does not seem responsible for the wilting.

(3) Ammonia and oxalate are produced by the fungus during its growth, but neither is present in sufficient quantity to explain the toxic action of the solution.

(4) The unknown substance responsible for the wilting is thermostable and non-volatile.

Since the above was written, a paper by Mr. Royal J. Haskel on "Fusarium wilt of potato in Hudson River Valley, New York," has come to my notice (Phytopathology 9: 223-260, 1919).

This writer describes certain experiments, which lead him to the conclusion that the wilting effect is not due to mechanical stoppage of the water-conducting vessels, but can be explained partly by the introduction of toxic substances into the transpiration current. His experiments suggest that these toxic substances may arise as decomposition products of the host tissue, as well as perhaps by actual secretion on the part of the fungus.

AN EFFECTIVE METHOD OF INOCULATING BARLEY WITH COVERED SMUT

W. H. TISDALE¹

The uncertainty of infection of barley by covered smut, *Ustilago hordei* (Pers.) Kell. and Sw., when the seed is smutted artificially has caused considerable concern among those investigating varietal resistance of barleys to this disease. Jensen² recognized this difficulty of infection in oats and barley as early as 1877 while attempting inoculations in his study of the specific differences between the smuts of these grains. He made the following statement:

"Since we have seen that spores simply adhering to the exterior of the grain do not to any appreciable extent cause infection of the crop it follows that this must take place by means of those spores which succeed in entering the space between the 'cosh' or husk and the kernel."

Jensen believed that infection in oats and barley came from spores which had lodged between the glumes at flowering time. The specific difference of the smuts of these grains, however, had not been determined definitely at that time and some doubt remained as to which of the two smuts of barley Jensen was using. In 1887, he removed the hulls (glumes) from some oat seed and dusted oat smut spores on the seed before sowing. Of the 33 heads produced, four were smutty, while no smut occurred either in plants from seed inoculated with the hulls on or from hulled or dehulled seed uninoculated.³ In 1888, in a more comprehensive experiment, Jensen found that oat seed, when dehulled and inoculated, produced 21 per cent of smutty heads, while dehulled seed, uninoculated, produced smut-free plants. Dehulled and inoculated barley seed produced plants with 27 per cent of smutty heads, while plants from dehulled seed uninoculated were smut-free. He dipped oat seed in spore-charged water and the plants grown from this seed produced 29 per cent of smutted heads, while seed dusted with spores produced smut-free plants. It is not known which smut of oats Jensen used in his oat experiments.

It is generally reported that naked (hull-less) barley and oats show more smut than do the varieties with the glumes closely adhering to the

¹ The writer is indebted to Mr. J. W. Taylor of the Office of Cereal Investigations, who furnished seed for these investigations.

² Jensen, J. L. The propagation and prevention of smut in oats and barley. Jour. Roy. Agric. Soc. 24: 397-415. 1888.

³ In this paper, "hulled seeds" are those in the natural state, with the hulls present; "dehulled seeds" are those with the hulls removed.

seed. In view of this fact and the results obtained by Jensen an attempt was made to determine by experiment the importance of the glumes in preventing infection by spores dusted on the hulled seed. In January, 1921, seed of a number of varieties of barley was sown in the greenhouse after dusting thoroughly with spores of covered smut. The resulting plants were free of smut. On November 1, 1921, seed of Tennessee Winter barley from a field practically free from covered smut was dehulled and inoculated and sown along with hulled seed which was similarly inoculated. The hulls were removed by breaking the basal or rachis end of the glumes from over the embryo and removing the remaining parts with a knife or scalpel. The results of this experiment are shown in table 1.

TABLE 1—*Infection, by covered smut, of Tennessee Winter barley grown from hulled and dehulled seed which was dusted with smut spores and sown in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., on Nov. 1, 1921.*

Treatment	Number of heads	Smutted heads	Per cent smutted
Dehulled seed; inoculated	461	392	85 03
Hulled seed; inoculated	403	16	3 97
Hulled seed; uninoculated	357	1	28
Hulled seed; covered with soil mixed with smut spores	376	10	2 66

The data in table 1 show that removing the hulls was very effective in producing infection in this particular case. Covering the hulled seed after sowing with soil heavily charged with spores had about the same effect as dusting the spores on the hulled seed.

In the fall of 1922, seed of 12 varieties of barley was sown in the greenhouse in a similar experiment. The seed was taken from the 1922 crop grown on Arlington Experiment Farm and some of it was known to be infested with covered smut. This smutted seed was used in order that natural inoculation might be compared with artificial inoculation. The results are shown in table 2.

The data in table 2 show that barley is much more subject to infection by covered smut if the hulls are removed before the seed is inoculated. There are some indications that varieties differ in their susceptibility to this smut. Nakano Wase failed to become smutted under any condition. Tennessee Winter grown from seed dehulled and uninoculated showed slightly more infection than plants from hulled seed which

TABLE 2—*Infection of 12 varieties of barley by covered smut after the seed was inoculated in various ways and sown in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., on October 21, 1922*

Variety	C. I. No.	Treatment	Number of plants	Per cent smutted plants	Number of heads	Per cent smutted heads
Alaska	534	Hulled, uninoculated	27	18 52	173	10 98
		Dehulled, inoculated	29	62 07	115	61 74
		Hulled; inoculated	25	12 00	156	13 46
Cartouch	1107	Hulled; uninoculated	22	0	149	0
		Dehulled; inoculated	14	85 71	77	74 02
		Hulled; inoculated	22	4 54	129	77
Cusado	895	Hulled; uninoculated	27	11 11	121	14 05
		Dehulled; inoculated	26	57 69	141	46 80
		Hulled, inoculated	29	3 45	128	2 34
Greece	221	Hulled; uninoculated	31	0	125	0
		Dehulled; inoculated	24	79 16	90	56 66
		Hulled; inoculated	27	3 70	126	1 58
Han River	206	Hulled; uninoculated	26	0	156	0
		Dehulled; inoculated	26	73 07	169	61 53
		Hulled; inoculated	25	4 00	173	3 46
Nakano Wase	754	Hulled; uninoculated	not counted	0	not counted	0
		Dehulled; inoculated	do	0	do	0
		Hulled; inoculated	do	0	do	0
Orel	351	Hulled; uninoculated	25	0	116	0
		Dehulled, inoculated	27	37 03	107	57 01
		Hulled; inoculated	28	0	215	0
Scottish Pearl	277	Hulled; uninoculated	25	8.00	186	9.14
		Dehulled; inoculated	22	68.18	177	67 89
		Hulled; inoculated	21	4.76	188	.53
Tenkau	646	Hulled; uninoculated	21	14.28	170	5 88
		Dehulled; inoculated	24	62.50	169	66.27
		Hulled; inoculated	23	4.34	175	1.14

Variety	C. I. No.	Treatment	Numbers of plants	Per cent smutted plants	Number of heads	Per cent smutted heads
Tennessee Winter	257	Hulled, uninoculated	25	8 00	254	1 96
		Dehulled, uninoculated	22	9 09	191	6 80
		Hulled; grown in greenhouse; uninoculated	25	0	209	0
		Hulled seed; grown in greenhouse; flowers inoculated	23	0	197	0
		Dehulled; inoculated	21	76 19	164	51 22
		Hulled, inoculated	24	25 00	190	10 52
		Hulled; covered with spore-charged soil	26	11 54	195	4 10
Texas Winter	554	Hulled; uninoculated	22	40 90	170	26 47
		Dehulled; inoculated	14	92 82	129	90 62
		Hulled; inoculated	23	4 34	173	1.73
Wisconsin Winter	519	Hulled; uninoculated	24	12 50	182	3.84
		Dehulled; inoculated	18	50 00	125	31 20
		Hulled; inoculated	22	4 54	170	.58

were uninoculated. This seems to indicate that the spores which infect are carried between the hulls and the kernel. However, plants grown from seed inoculated by dusting spores between the glumes at flowering time showed no smut. The high temperature existing in the greenhouse where these inoculations were made might have affected the results. Covering the seed with spore-charged soil after sowing failed to increase the percentage of infection materially.

The sowings listed in table 2 were repeated in the field at Arlington Experiment Farm, but no results were obtained, due to almost complete winter-killing of plants from dehulled seed, which was sown very thinly. If a more efficient means of dehulling the seed could be found or if the inoculation could be accomplished in some other way with less trouble the testing of barleys for resistance to covered smut could be accomplished with less delay. Possibly scarifying the seed before smutting would give the same results as the method of dehulling employed. The hulls seem to be the main obstacle in the way of successful inoculation.

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SOME POSSIBLE RELATIONSHIPS OF THE MOSAIC DISEASES

H. A. ALLARD

A mass of published material has accumulated dealing with the results of observations and experiments bearing on the mosaic diseases of the potato, tobacco, and likewise of other plants outside of the Solanaceous Family. A great deal has been learned, but much still remains to be learned concerning all these diseases. Now and again it is a good thing to emphasize what we do not know as well as to keep in mind what we really know concerning any problem. Perhaps it would be just as well if we published our lack of knowledge occasionally, for the time completely divorcing ourselves from favored theories and orthodoxies which may have been unduly emphasized in our papers dealing with positive results.

Let us consider some angles of the problems connected with the mosaic diseases of the Solanaceous plants. The following points have not yet been cleared:

(1) The nature of the infective agent has not been determined, whether bacterial, protozoan, etc.

(2) The actual relation of the mosaic disease of potatoes to the mosaic disease of tomatoes and tobacco is not known; whether or not the two are specifically distinct, or simply due to physiological variations in a common infective agent.

(3) It is not known just how the "virus" is carried over the winter in all instances. Affected perennials such as species of *Physalis*, and *Solanum carolinense* are known to be one factor in the case of the mosaic disease of tobacco and tomatoes. A second and perhaps very important reservoir of the virus may be overwintering plant lice or other insects, but this has not been established. If the virus is carried over the winter by insects, there are two possibilities, one the mere mechanical transfer of the virus through infected mouth-parts, etc., of overwintering insects which have fed upon mosaic diseased plants. In the case of certain plant lice which are known to be active carriers of the virus, it is also possible that a more complex relationship exists between the infective agent and the aphid. Several nice points are involved here. It has already been shown that certain species of plant lice when allowed to feed upon mosaic diseased tobacco, tomato, or potato plants are able to transfer the infective agent readily to healthy plants of the same kind. Is it necessary for each generation to pick up the infective agent anew, or is it possible for the viviparous females to transmit the infective agent to their living

progeny? Finally, is it possible for the infective agent causing these diseases to be transmitted to the overwintering eggs produced by the last generation of the season, ultimately to reappear in the stem mothers and successive generations as a source of infection for the new crop of tobacco, tomatoes, and potatoes? If plant lice which appear to be the natural carriers of the virus of the mosaic diseases serve to carry the virus over the winter, some such method must obtain, and it becomes a question of great importance to establish the truth of these complicated relations. Both phases of the question are equally important since in colder climates the link may be dependent upon the overwintering egg alone, or in the warmer portions of the country the link may be dependent upon overwintering viviparous females themselves. The transfer of the "virus" from generation to generation through viviparous females, or through the egg becomes a more involved relation of a more or less symbiotic nature. There is nothing paradoxical in the possibilities of such relations, since it is already established that very complicated relations may obtain between various disease producing organisms and their alternative insect and animal hosts. The South African Coast Fever is one of these in which the Brown Tick (*Eurhipicephalus appendiculatus*) spreads the blood parasite (*Babesia parva*) among cattle and buffaloes. The life history and host relations are briefly these: The ticks lay their eggs in the grass, these hatching in about 28 days into 6-legged larvae which attach themselves to passing cattle, feeding upon their blood. These fall off in three or four days, remaining dormant about 21 days, then moult, giving rise to 8-legged nymphs. These again pass to cattle, sucking their blood for the same length of time as the larvae, finally dropping off replete, moulting in about 18 days and becoming adult male or female ticks. These attack cattle, mating takes place and egg-laying in the grass completes the cycle of the tick carrying the protozoan parasite. Strange as it may seem only the nymphs in this cycle transmit the disease-producing parasite (*Babesia parva*) to healthy animals although the larvae and adults themselves suck blood as well. Moreover, the larvae, which do not themselves transfer the disease, yet retain the infective agent which appears in an actively infecting form in the nymphs themselves. Here, if the facts are correctly worked out, we have an intermediate stage alone in the life cycle of the tick-host capable of transferring the disease, with blood-sucking stages on either side, the larvae on one, the adults on the other, neither of which transfers infection.

While it has been established without much question that the mosaic "virus" may overwinter in certain perennial plants in some instances.

this does not always appear to account for the sudden appearance and spread of the disease in all localities. The missing link that completes the story may well be an overwintering of the "virus" in the bodies or in the eggs of certain plant lice which are active carriers of infection. The problem is an interesting and important one and may clear up the question of the overwintering of some reservoir of infection at the present time not clearly understood.

Theories in themselves are of no value since they are but tentative explanations of conditions and relations not completely understood. Theories, nevertheless, have their place, their rôle, since they may serve as a vantage point from which to lead out in a problem where real work is being followed. A theory at best is a temporary attitude of the mind and new facts may support or destroy it. A theory becomes old and useless when experience has progressed beyond its conditions and limitations. In any problem involving such intricacies of relationship as the mosaic diseases which include such possibilities as an infectious "virus" carried by particular insects, particular species of plant lice, overwintering in affected perennials, and perhaps in the insect carriers themselves or their eggs, other possibilities of the problem should not be slighted or ignored. The forces of life have developed many strange and incomprehensible relationships as exemplified in the living relationships of hosts of organisms. There is one question that has come to my mind on several occasions which may at first seem fanciful, yet nevertheless in it rests a remote possibility, and no possibility is so remote that it can not bear some relationship to reality. Many fungus and bacterial diseases affect plants, and tobacco, tomatoes, and potatoes are no exceptions. Is it possible for the bacterial cells or the spores or hyphae of invading fungi to become contaminated with the "virus" or actually to absorb or include the infective principle in mosaic-diseased tissues, thus transferring infection in some instances or even serving as an overwintering host? The fact that certain species of fungi and bacteria are so nicely related physiologically to certain host plants as to find their nutrition solely within the sap, tissues, and protoplasm of the latter would suggest that relationship might even exist between some of the "virus" diseases and these more evident, more visible parasites, both of which have become more or less highly adapted to the same physiological environment. This is merely a suggestion, but while we are looking so strenuously for an overwintering reservoir for the "virus" in some of the higher plants or insects, it may be just as logical to eliminate some of the possibilities lower down in the scale among invading bacterial and fungus parasites which may at times become intimately associated with the "virus."

BUREAU OF PLANT INDUSTRY,

U. S. DEPARTMENT OF AGRICULTURE.

A METHOD OF ISOLATING SELECTED SINGLE SPORES

JOHN W. ROBERTS

WITH ONE FIGURE IN THE TEXT

In connection with a series of experiments involving spore-selection, it became necessary to isolate not only single spores but also certain individual spores selected from a large number. After selection and isolation the spores were transferred to artificial media for the purpose of permitting study of the resulting growth and further selection from another generation.

The method used was a modification of that employed by Edgerton.¹ The apparatus designed by Edgerton consisted of a capillary tube fastened in an ordinary rubber-tube clamp mounted on a cork which was fitted into the diaphragm opening of the substage condenser of a microscope in the "swung out" position. Orientation of the open end of the capillary tube, which was simply a tube of a very small bore drawn to a capillary point at one end and closed at the other, was accomplished by turning the whole apparatus and by raising and lowering it by means of the screw which raises and lowers the condenser. The spore and a drop of the water surrounding it were drawn into the tube by applying ether to the closed end and expelled by warming the closed end. The spore was usually forced out upon a sterile cover slip, examined under the microscope and if satisfactory the slip with the drop containing the spore was pressed into media contained in a Petri dish. Observation of the spore was also made during germination to make sure that only one had been picked up.

As the rubber-tube clamp with its elastic-band fastening used by Edgerton to hold the capillary tube was not sufficiently sturdy to give the rigid support desired, a holder was devised to meet this need. A support (Fig. 1, a) commonly found on ring stands and used to hold test tubes or flasks was unscrewed from the clamp holding it to the upright of the ring stand and mounted on the cork by simply twisting the threaded end through the center of the cork. The jaws of the support were lined with cork (b) so that when closed they fitted tightly together save for a groove into which the capillary tube (c) was fitted.

When using the apparatus, the capillary tube (c) is first adjusted so that when swung into position beneath the objective of the microscope, the open end is seen near the center of the field. By turning the entire apparatus the tube is then swung to one side and adjusted to a height

¹ Edgerton, C. W. *Phytopath.* 4: 115-117. 1914. 1 fig.

such that when swung back into position it clears the surface of the stage by several millimeters. The slide with a comparatively small number of spores mounted in water is now placed in position and the spore having the desired characters is selected and placed near the center of the field by manipulating the slide. The apparatus is then swung about so that the open end of the capillary tube appears in the

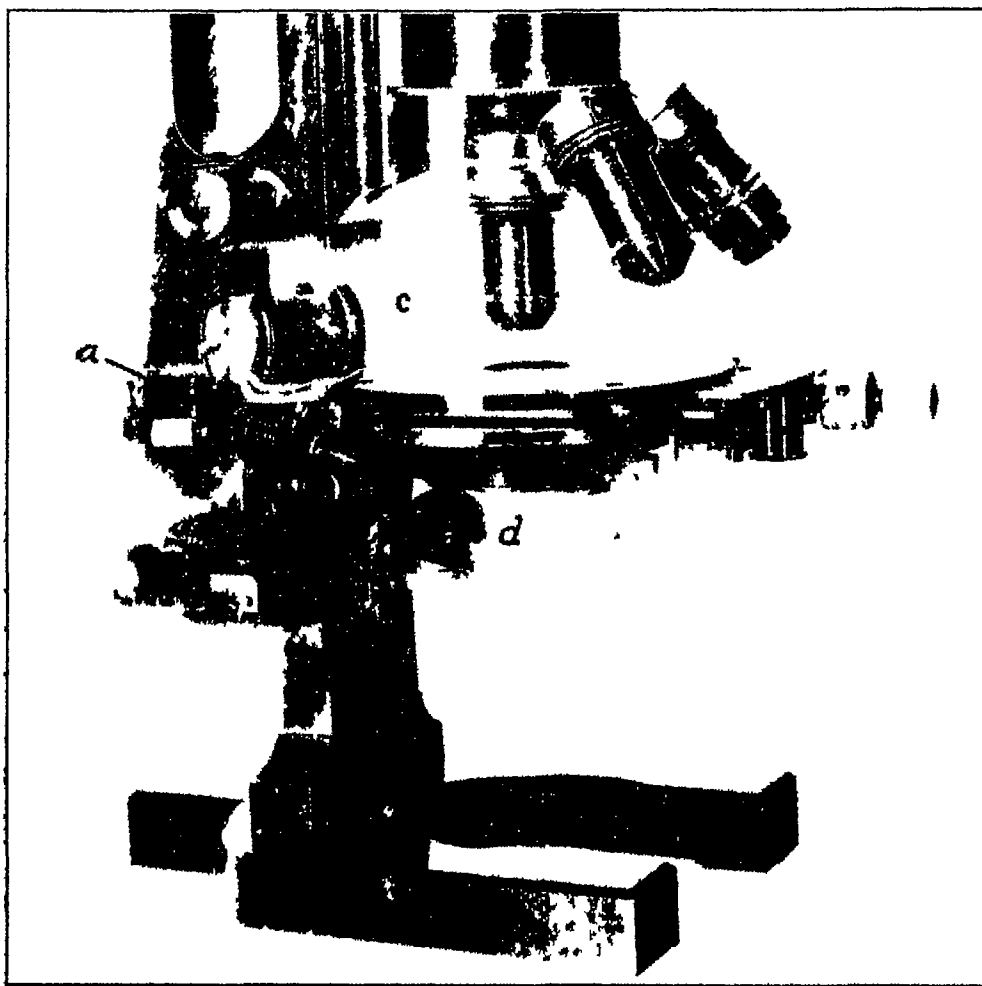


FIG. 1 Apparatus for picking up single spores

center of the field almost directly above the spore. By means of the lowering screw (d) the end is then lowered until it strikes the water. Sometimes the spore is sucked up immediately but more often it is necessary to increase the suction by applying ether to the closed end of the tube. After the spore in a small amount of water has been taken up, the capillary tube is raised from the water by means of the screw and the whole apparatus is swung to the side. After careful removal of the capillary tube from its support, the closed end is held over a low flame and the open end over artificial media contained in a Petri dish.

By this means the drop of water containing the spore is driven out of the tube and is made to fall into the Petri dish within the circumference of a circle about 3 mm. in diameter previously indicated by marks on the bottom of the dish. As many as a dozen such transfers may be made to one Petri dish. The flame used should be a very small one, else the water in the tube may become too hot or may be changed to vapor. A flame of the small burner known as the "Micro Burner" has been very satisfactory.

As the circles indicate the location of each drop, the Petri dish is placed under the microscope and the contents of the drops examined to be sure that not more than one spore was picked up and to be sure that the one picked up is the one wanted. Another examination is made after germination has begun, at which time every spore is easily located. If two spores should be found in a single drop, growth from that drop may be discarded. Often, however, they are so far apart that their positions can be indicated by marks made on the under side of the Petri dish. The desired spore can then be transferred to another Petri dish or tube of media.

Special attention should be paid to Edgerton's instructions regarding capillary tubes. He advises that they be provided in advance, placed in plugged test tubes and sterilized. He also states that they should be made from as small glass tubes as possible, not larger than 2 mm. diameter inside. In a smaller tube there is less volume of air and the expansion and contraction of this is less, making manipulation easier. Also a smaller tube can be drawn out more satisfactorily than a larger one. To Edgerton's remarks, it should be added that the open end of the tube should be but little wider than is necessary to admit a single spore. With large spores it is possible to have the opening too small, but with smaller spores it is often too large.

To obviate the necessity of alternately turning out and lighting the flame, the ether is kept in a small-mouthed bottle, in the stopper of which a small glass rod is inserted. By this arrangement a drop may be placed upon the closed end of the tube with the release of a minimum quantity of ether fumes into the room.

The method here given may, at times, seem laborious and unduly tedious but by its use one can be absolutely certain that one has isolated not merely a single spore but a particular single spore possessing those characters which he wishes to study. The rigidity of the apparatus here described, permitting of delicacy and accuracy of operation, greatly facilitates the task.

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PHYTOPATHOLOGICAL NOTES

Foot-rot or Ophiobolus in California. —For three or four years the search for foot-rot (*Ophiobolus graminis*) in the grain fields of California failed to positively identify this disease. However, during the past season there appeared in the plots of wheat at Davis plants which appeared to be attacked by *Ophiobolus*. These plots were irrigated every week during the last four or five weeks of the growing season in order to induce stem rust attack. As the plants came into head the spikes appeared light in color and weight and did not bend under the weight of the filling head. Hormodendron lesions appeared on the glumes as small black blotches. The kernels when examined were found in every case to be badly shriveled and useless for milling or seeding purposes. The plants when pulled came out of the soil readily. The roots were decidedly gray in color and had broken off short where rotting had set in. Enlargement of the roots of diseased plants was common. Such enlarged roots (almost three or four times the normal diameter) when cross-sectioned were found to be hollow throughout the length of the enlarged portion.

The culms were usually weak and looked like those of prematurely ripening plants. In number the culms were not reduced below that for disease-free plants.

Upon the surface of the culm on the first and second internode, usually below the surface of the soil, appeared blackened areas, with lighter areas between. Upon the inner surface of the enveloping leaf sheath appeared black bodies which on examination proved to be perithecia filled with asci containing eight ascospores. The spores were extremely long and linear in shape. The disease was definitely identified as *Ophiobolus graminis* by Dr. H. B. Humphrey.

Specimens collected by the author for the past four years over California indicate that the disease is widespread and apparently has been established in the State for many years and does not appear to be increasing. Foot-rot has been found to cause blighted heads in wheat fields to the extent of 15 per cent of the crop.

Hitherto the specimens observed have failed to show perithecia which made certain identification impossible. The blackened area appeared on the surface of the internode as usual and the mycelial web or blackened area extended to the inner surface of the enclosing sheath, but no perithecia appeared.

It is believed that the application of irrigation water late in the season gave the *Ophiobolus* the proper conditions required for the development of perithecia.

At Davis, *Ophiobolus* perithecia were this year found on barley. Mr. McKinney in 1922 reports that he found barley attacked west of Riverside in Southern California.

So far as the observations have extended no variety of wheat has been

found immune from attack, but certain varieties like Hard and White Federation appear peculiarly susceptible.—W. W. MACKIE.

A new disease of cultivated barley in California caused by Helminthosporium californicum, n. sp.—An undescribed species of Helminthosporium has been observed to be generally distributed and abundant in California on cultivated barley. Its period of development begins much later than that of other Helminthosporium species previously described. It appears with the warm, dry weather and develops rapidly. The apical ends of the leaves are first attacked and finally a "rusty blotch" is formed on the leaves. The lower leaves are first attacked and the disease progresses to the top of the plant. The grain is shriveled due to the premature death of the leaves. The spots are irregular, brown to bluish black, usually merging into diffused brownish discoloration of larger part of leaf. The spots are not netted (*H. teres*), elongated (*H. gramineum*), or definitely bounded dead areas (*H. sativum*).

The conidia of *H. californicum* n. sp., are dark olivaceous when fully mature; fusiform, not curved; thick walled; $40-85 \mu \times 20-26 \mu$; average $58 \times 22 \mu$; 3-9 septate. Germinate by single germ tube from any segment.

Resembles *H. sativum* P. K. & B., except that it occurs later, is more severe, the diseased areas are not definite and have no distinct margins. The conidia are longer, wider, more dense, thicker walled and not curved.

The rusty blotch which has been observed for four years in California has been determined to be a new species of Helminthosporium and has been named *H. californicum*. It has been increasing in severity in the Sacramento and San Joaquin Valleys. Studies carried on show marked varietal resistance to this disease.—W. W. MACKIE AND G. E. PAXTON.

Personals—Dr. George H. Pethybridge, formerly Head of the Seeds and Plant Diseases Division of the Department of Agriculture and Technical Instruction for Ireland, has been appointed Mycologist to the Ministry of Agriculture and Fisheries for England. His headquarters will be at Harpenden.

Dr. Paul A. Murphy has been appointed Head of the Plant Diseases Division of the Department of Agriculture and Technical Instruction for Ireland. Dr. Murphy, whose address is Plant Diseases Division, College of Science, Dublin, writes to say that he wishes to keep up close coöperation with his American and Canadian colleagues and would be glad if individuals and institutions which formerly supplied publications of a pathological nature to the Seeds and Plant Diseases Division would send them personally to him.

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